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How Bad Could It Be? Alcohol Dampens Stress Responses to Threat of Uncertain Intensity

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Abstract
Stress response dampening is an important motive for alcohol use. However, stress reduction via alcohol (alcohol SRD) is observed inconsistently in the laboratory, and this has raised questions about the precise mechanisms and boundary conditions for these effects. Emerging evidence indicates that alcohol SRD may be observed selectively during uncertain but not certain threats. In a final sample of 89 participants, we measured stress response via potentiation of defensive startle reflex in response to threat of shock in blocks with certain (low and high) and uncertain shock intensity. Our alcohol-administration procedure produced blood alcohol concentrations (BACs) across a broad range (0.00%–0.12%) across participants. Increasing BACs were associated with linearly decreasing startle potentiation and self-reported anxiety. This SRD effect was greater during uncertain than certain threat. More broadly, these results suggest that distinct mechanisms are involved in response to threats of uncertain intensity and threats of certain intensity.

Keywords
drug and substance abuse, stress reactions, emotions, startle reflex

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> Imagine that you have arrived early to a restaurant, with the intention to break up with your significant other. You know this conversation will be stressful, but you cannot anticipate how strongly your partner will react. You could be met with any reaction ranging from uncomfortable silence to loud shouting and crying. You order a large glass of wine to steady your nerves in response to this uncertainty.

Will this glass of wine have the desired effect? Drinkers expect alcohol to reduce their negative affective response to stressors (Sher, 1987). Furthermore, both recreational and problem drinkers report that stress reduction is an important motive for their alcohol use (Cooper, 1994; Schroder & Perrine, 2007). However, three decades of research have yet to specify the precise mechanisms and boundary conditions for stress response dampening via alcohol, or *alcohol SRD* (Levenson, Sher, Grossman, Newman, & Newlin, 1980; Sher, 1987; for a review, see Curtin & Lang, 2007). Researchers need to clarify when, how, and for whom alcohol SRD occurs to answer fundamental questions about this popular drug's reinforcing effects and to improve treatments for its excessive use. In addition, answers to these questions promise to advance understanding of the psychological and neurobiological mechanisms involved in the affective response to stressors more generally.

Uncertain Versus Certain Threat

The opening scenario exemplifies anticipation of an uncertain stressful encounter. Stressors vary on several dimensions of threat uncertainty. For example, threats or other stressors can be probabilistically and temporally uncertain. Affective scientists have developed tasks to examine the mechanisms that mediate the impact of uncertainty about threat probability (*if* the threat will occur) and threat onset (*when* the threat will occur) in humans and animal models (Davis, Walker, Miles, &

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Grillon, 2010; Mobbs et al., 2007). Multiple lines of evidence suggest that these uncertain threats can be distinguished from certain threats by the emotional, behavioral, and cognitive-attentional responses they elicit, the time course of these responses, and the neural mechanisms mediating these responses (Davis, 2006).

For instance, uncertain and certain threats produce distinct emotional responses characterized in humans as anxiety in the former case and fear in the latter (Grillon, 2008). Uncertain threats elicit freezing and hypervigilance in animals, whereas certain threats elicit active avoidance, defensive attack, or both (Blanchard & Blanchard, 1989). Imminent, certain threats focus attention on the threat itself, whereas distal, temporally uncertain threats encourage distributed attention to the overall environment (Cornwell, Echiverri, Covington, & Grillon, 2008; Fanselow & Lester, 1988; Mobbs et al., 2007). Response to temporally uncertain threats appears to be sustained, whereas response to certain threats is phasic and time-locked to the threat (Davis et al., 2010). Finally, neuroscience research with rodents has shown that a pathway involving the lateral divisions of the central nucleus of the amygdala and the bed nucleus of the stria terminalis appears to selectively mediate sustained response to temporally uncertain threats, perhaps through sensitivity to corticotrophin-releasing factor and norepinephrine (Walker & Davis, 2008).

Grillon and his colleagues have manipulated uncertainty about if and when threats will occur in humans using their no-shock, predictable-shock, unpredictable-shock (NPU) task (Schmitz & Grillon, 2012). Predictable and unpredictable shock both potentiate the startle reflex, a cross-species physiological index of defensive reflexive responding. Patients with posttraumatic stress and panic disorders exhibit selectively increased startle potentiation during unpredictable but not predictable shock in the NPU task (Grillon et al., 2008; Grillon, Pine, et al., 2009). Medications prescribed to treat anxiety appear to have greater effect on startle potentiation during unpredictable shock than during predictable shock in the NPU task (Grillon, Chavis, Covington, & Pine, 2009).

Most research on alcohol SRD has not included attempts to vary threat uncertainty. However, Moberg and Curtin (2009) demonstrated that a moderate dose of alcohol (approximately four standard drinks over 1 hr in a 180-pound man) selectively reduced startle potentiation during threat of unpredictable but not predictable shock in the NPU task. In two follow-up experiments, Curtin and his colleagues confirmed that greater alcohol SRD during uncertain than during certain threat was observed with more precise, separate manipulations of expectations regarding if (probabilistic uncertainty; Hefner & Curtin, 2012) and when (temporal uncertainty; Hefner, Moberg, Hachiya, & Curtin, 2013) the threat would occur.

If, When, and How Bad?

Initial basic affective science and our laboratory's research on alcohol SRD have focused on uncertainty regarding if and when threats will occur (Davis et al., 2010; Hefner & Curtin, 2012; Hefner et al., 2013; Moberg & Curtin, 2009). It is now time to probe the boundary conditions of uncertainty to better define this construct. In the opening scenario, you are relatively certain about if and when your partner's reaction will occur. The uncertainty is constrained primarily to the severity dimension (i.e., how bad the reaction will be). In the experiment reported here, we followed emerging interest in this understudied dimension of threat uncertainty (Shankman, Robison-Andrew, Nelson, Altman, & Campbell, 2011) to test the novel prediction that alcohol SRD will be more robust when threat severity is uncertain than when it is certain.

Alcohol Dose Response and Uncertain Threat

In the opening scenario, you might expect the amount of wine to influence its effectiveness. In fact, Sher (1987) found that alcohol SRD was consistently observed with intoxicating doses of alcohol. Thus, initial theoretical accounts predicted that alcohol dose would moderate the magnitude of alcohol SRD but did not specify the form of the dose response function (e.g., linear, threshold, or asymptotic). However, only a handful of experiments on alcohol SRD have examined alcohol dose response (Donohue, Curtin, Patrick, & Lang, 2007; Moberg, Weber, & Curtin, 2011; Sher & Walitzer, 1986; Stewart, Finn, & Pihl, 1992). Furthermore, these experiments included no more than three active doses, and therefore did not allow clear specification of the dose response function (but see Moberg et al., 2011). None of these studies compared dose response effects on alcohol SRD during uncertain versus certain threats of any kind.

The Current Study

We manipulated threat uncertainty and severity by exposing participants to blocks of certain low-intensity shock, certain high-intensity shock, and uncertain-intensity shock. We administered various doses of alcohol to manipulate participants' blood alcohol concentrations (BACs) quantitatively across a broad range of ecologically meaningful levels from sobriety to moderately high intoxication (approximately six standard drinks over 1 hr in a 180-pound man). We assessed participants' stress response by measuring the potentiation of their defensive startle reflex during the threats. We tested the following two predictions: First, we expected alcohol SRD to be dose dependent, with increasing SRD (i.e., decreasing

startle potentiation) associated with increasing BAC. Second, we expected alcohol to produce selectively greater SRD during threat of uncertain-intensity shock than during threat of certain-intensity shock. We included two threats of certain intensity to evaluate a competing hypothesis that the magnitude of alcohol SRD varies with the intensity of the stress response rather than the uncertainty of the stressor. We conducted supplemental analyses to examine potential individual difference moderators of alcohol SRD. Finally, we included a measure of self-reported anxiety to index participants' subjective emotional response to the threats of certain and uncertain intensity.

Method

Participants

We recruited 89[1] participants (45 female, 44 male; mean age = 21.7 years, *SD* = 1.2 years) from the university community. All were at least 21 years old, had experience with the highest study dose of alcohol within the past year, and reported no history of alcohol-related problems (CAGE questionnaire; Mayfield, Mcleod, & Hall, 1974), no current use of psychiatric medication, and no medical condition that would contraindicate alcohol consumption. No participants were pregnant (verified by urine sample). Participants were instructed to abstain from alcohol and other drugs for 24 hr and from all food and beverages except water for 4 hr prior to their experimental session. We verified that all participants were sober on arrival via breathalyzer (Alcosensor IV; Intoximeters Inc., St. Louis, MO). We paid participants $10 per hour or class extra-credit points.

Baseline startle assessment

Participants' baseline startle response to acoustic startle probes was assessed in a pretask procedure during which they viewed a series of 12 colored squares presented on a CRT monitor for 5 s each with a variable intertrial interval (ITI; range = 10–20 s). Baseline startle response was included in analyses as a covariate to control for individual differences in startle potentiation (Hefner & Curtin, 2012; Hogle, Kaye, & Curtin, 2010; Moberg et al., 2011). (See the Supplemental Material available online for details on measurement of the startle response in the baseline and main tasks.)

BAC manipulation

Approximately equal numbers of male and female participants were randomly administered each of 12 alcohol doses (placebo and doses with target BACs from 0.01% to 0.11%, in increments of 0.01%). We assigned an additional 6 participants to both the placebo and the 0.11%-dose conditions to increase power. All participants were informed that they would receive a moderately impairing dose of alcohol that should produce a BAC of approximately 0.08%.

The alcoholic beverages consisted of 100-proof vodka (Smirnoff Blue Label), water, and a juice mixer, with the juice accounting for three quarters of the drink volume. We calculated the alcohol dose to produce the target BAC approximately 30 min after beverage consumption (see Curtin & Fairchild, 2003, for details regarding the dosing formula). Participants assigned to the placebo group received a beverage consisting of fruit juice mixed with water poured from a vodka bottle in their presence (participants who received alcohol similarly saw the vodka poured from a vodka bottle in their presence). The total volume of all beverages was matched to the volume of the beverage for the 0.11%-BAC group, with water replacing the equivalent volume of alcohol. Outside of participants' view, all drinks were misted with alcohol, and 2 ml of alcohol was floated on top of the beverages to provide sensory stimulation to support the placebo manipulation. Each participant's beverage was divided into four drinks, each consumed over 10 min, for a total drinking period of 40 min. The experimental session began 15 min after the end of the drinking period. We measured BAC immediately before, at the midpoint of, and immediately after completion of the main task. We used mean achieved BAC (average BAC across the three assessment times) in analyses evaluating alcohol's effects on startle potentiation.

Assessment of subjective shock tolerance

We measured participants' subjective shock tolerance following standardized procedures from our laboratory (e.g., Curtin, Patrick, Lang, Cacioppo, & Birbaumer, 2001; Moberg & Curtin, 2009). Five minutes after the drinking period, participants reported their response to a series of 200-ms electric shocks of increasing intensity. Shocks were administered across the distal phalanges of the index and ring fingers of the left hand. The procedure was stopped once participants reached the maximum level of shock that they could tolerate.

Cued-threat task

The main task was a cued-threat task consisting of eight blocks. In each block, participants viewed a series of five colored square cues that were presented on a CRT monitor for 5 s each and separated by a variable ITI (10–20 s, *M* = 15 s). We measured startle response to acoustic startle probes presented five times per block (three during cues, two during ITIs). There were four block types:

certain low-intensity shock, certain high-intensity shock, uncertain-intensity shock, and no shock. We instructed participants that shocks at the indicated intensity level would be administered 4.8 s after onset of each cue during all shock blocks and that no shocks would be administered during any ITI or at any time in no-shock blocks. We set the intensity levels for the low-shock and high-shock blocks, respectively, to 33% and 100% of each participant's maximum reported subjective shock tolerance. In uncertain-intensity blocks, we instructed participants that shock intensity would vary across cues but would never exceed the intensity of shocks delivered in the high-intensity blocks. In fact, low- and high-intensity shocks were equiprobable, intermixed randomly across cues in uncertain-intensity blocks.

A preblock message on the monitor informed participants of the next block type. A two-character condition abbreviation was presented in the center of each cue to further reinforce condition information: "LO" during low-intensity blocks, "HI" during high-intensity blocks, "??" during uncertain-intensity blocks, and "NS" during no-shock blocks. We used three different block orders, which were counterbalanced across subjects. We scored mean startle potentiation (i.e., increase in startle response to acoustic startle probes during threat blocks relative to no-threat blocks) separately for the three threat types (uncertain intensity vs. high intensity vs. low intensity).

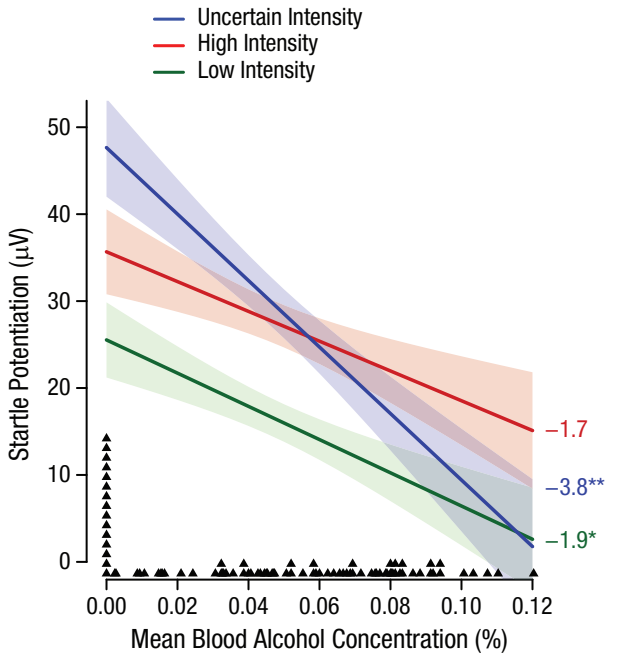

Fig. 1. Startle potentiation as a function of mean blood alcohol concentration (BAC) and threat type. The translucent bands indicate confidence envelopes (±1 *SE*) around the point estimates (dark lines) of mean startle potentiation from the general linear model. The strip plot (triangles) along the *x*-axis shows the observed mean BACs for all participants. The numbers in the right margin are coefficients from the general linear model, showing the simple effect of BAC for each threat type (*$p < .05$; **$p < .001$).

Post-threat-task measures

After the cued-threat task and final BAC assessment, participants rated how anxious they were when they saw each threat cue, using a 5 point rating scale (1 = *not at all anxious*; 5 = *extremely anxious*). They then completed self-report individual difference measures of personality and alcohol use. Finally, participants were debriefed, compensated, and dismissed once reaching a BAC below 0.03%.

Results

We analyzed data with R (R Development Core Team, 2013). The mean BAC for participants who were administered alcohol was 0.058% (*SD* = 0.03%) immediately before the main task, 0.059% (*SD* = 0.03%) in the middle of the task, and 0.059% (*SD* = 0.03%) immediately after the task. The strip plot in Figure 1 shows the mean BAC for all individuals.

Startle potentiation

We analyzed startle potentiation in a general linear model (GLM) with repeated measures for threat type (uncertain vs. high vs. low). Fully interactive between-subjects regressors for mean BAC, baseline startle, gender, and block order were included in the GLM.[2] We report unweighted BAC effects across the baseline-startle, gender, and block-order covariates. (The effects of these covariates and of individual difference moderators related to personality and alcohol use are discussed in the Supplemental Material). To test our predictions, we examined two planned orthogonal contrasts for threat type: (a) startle potentiation in uncertain-intensity blocks versus average startle potentiation across low-intensity and high-intensity blocks and (b) startle potentiation in high-intensity blocks versus startle potentiation in low-intensity blocks. We report raw GLM coefficients (*b*) and partial eta-squared (η_p^2) to document effect sizes.

Mean startle potentiation was significant (nonzero) across threat types at a BAC of 0.00%, $b = 36.2$, $t(65) = 9.10$, $p < .001$, $\eta_p^2 = .56$ (Fig. 1). Startle potentiation was increased significantly during uncertain-intensity threat compared with the average startle potentiation across certain high-intensity and low-intensity threat, $b = 17.1$, $t(65) = 3.69$, $p < .001$, $\eta_p^2 = .17$. In addition, startle potentiation was significantly increased during certain high-intensity threat relative to certain low-intensity threat, $b = 10.1$, $t(65) = 2.04$, $p = .046$, $\eta_p^2 = .06$. Despite differences

across threat type, startle potentiation was significant (nonzero) for each threat type (ps < .001).

As predicted, the overall effect of mean BAC across threat types was significant, such that startle potentiation decreased 2.5 μV for every 0.01% increase in BAC, b = −2.5, $t(65)$ = 3.48, p = .001, η_p^2 = .16. Also as predicted, interaction contrasts indicated that this mean BAC effect was significantly greater during uncertain threat (b = −3.8) than during certain (average of high and low) threat (b = −1.8), b = −2.0, $t(65)$ = 2.44, p = .018, η_p^2 = .08. In contrast, the magnitude of the mean BAC effect was comparable across certain high (b = −1.7) and certain low (b = −1.9) threat, b = 0.2, $t(65)$ = 0.22, p = .825, η_p^2 = .00.

Linearity of the BAC effect on startle potentiation

We assessed the linearity of the BAC effect on startle potentiation in two ways. First, via linear inspection, we confirmed that the patterns of residuals for the BAC effect in component-plus-residual plots were consistent with linear effects during both certain and uncertain threat (Fox, 2008); specifically, the residuals were symmetrically distributed around the BAC regression line for all BACs (Fig. 2). Second, we included regressors for higher-order (i.e., quadratic and cubic) BAC effects in supplemental GLMs. The effects of these higher-order regressors were not significant for either certain-threat contrasts (quadratic: p = .479; cubic: p = .408) or uncertain-threat contrasts (quadratic: p = .233, cubic: p = .484).

Self-reported anxiety in response to cues

We conducted analyses of self-reported anxiety during the cues in a GLM using the same model that we used to analyze startle potentiation. (We report the effects of the covariates from this analysis, as well as correlations between self-reported anxiety and startle potentiation, in the Supplemental Material.) At a BAC of 0.00%, self-reported anxiety was significantly greater during uncertain threat compared with the average anxiety during certain high and certain low threat, b = 0.84, $t(65)$ = 4.65, p < .001, η_p^2 = .25 (Fig. 3). Self-reported anxiety was also greater during certain high threat relative to certain low threat, b = 1.47, $t(65)$ = 7.23, p < .001, η_p^2 = .45.

The overall effect of mean BAC across threat types was significant, such that self-reported anxiety decreased 0.09 units for every 0.01% increase in BAC, b = −0.09, $t(65)$ = 3.06, p = .003, η_p^2 = .13. Interaction contrasts indicated that this mean BAC effect was significantly greater during uncertain threat (b = −0.14) than during certain (average of high and low) threat (b = −0.07), b = −0.07, $t(65)$ = 2.09, p = .041, η_p^2 = .06. In contrast, the magnitude of the mean BAC effect was comparable across certain high (b = −0.06) and certain low (b = −0.08) threat, b = 0.02,

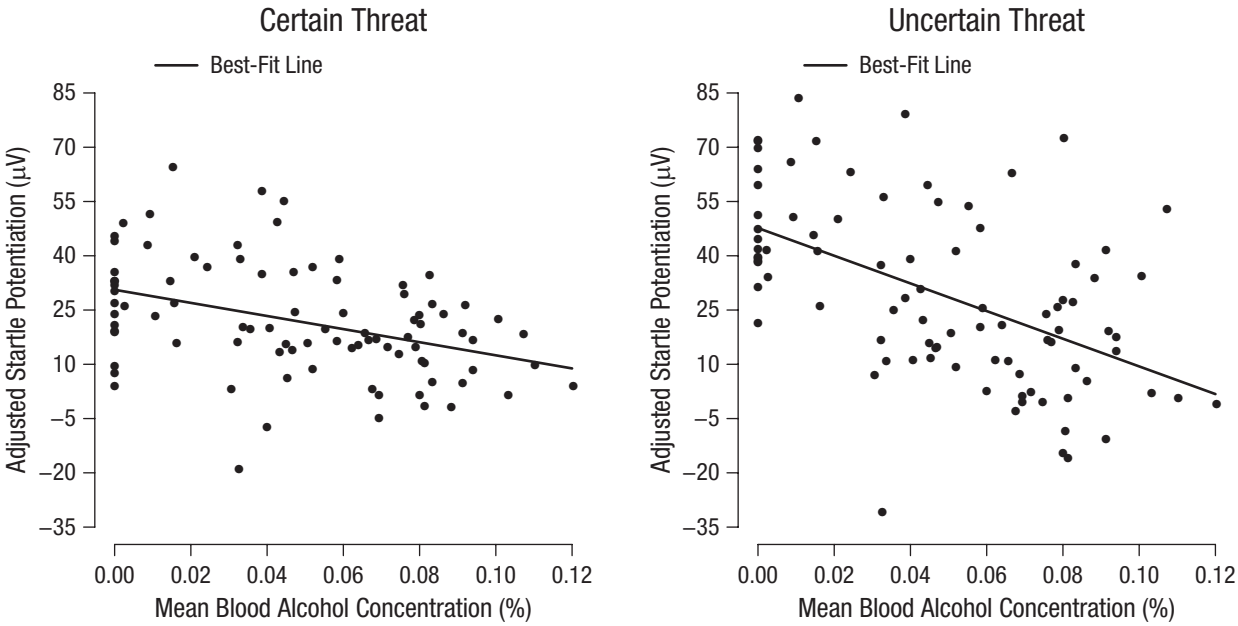

Fig. 2. Component-plus-residual plots for the effect of blood alcohol concentration (BAC) on startle potentiation during certain and uncertain threat. Component-plus-residual plots are used to assess linearity of effects in general linear models (Fox, 2008). Startle potentiation scores in these plots were adjusted to control for all regressors in the models other than BAC.

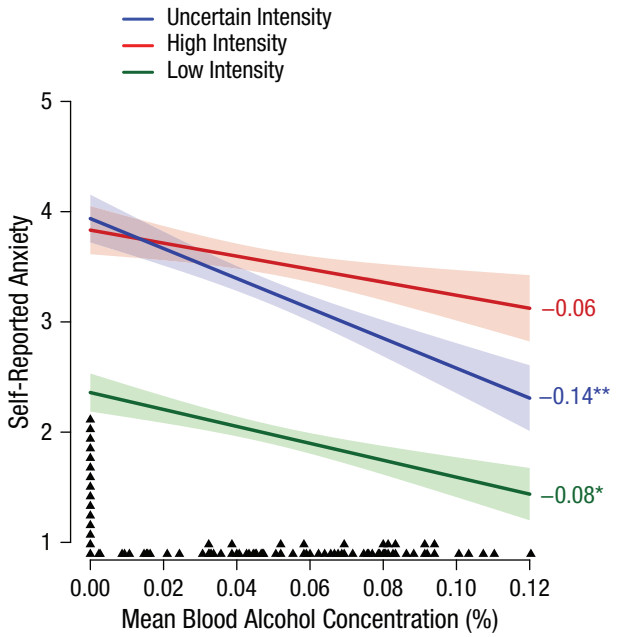

Fig. 3. Self-reported anxiety (1 = *not at all anxious*; 5 = *extremely anxious*) as a function of mean blood alcohol concentration (BAC) and threat type. The translucent colored bands indicate confidence envelopes (±1 *SE*) for point estimates (dark lines) of self-reported anxiety from the general linear model. The strip plot (triangles) along the *x*-axis shows the observed mean BACs for all participants. The numbers in the right margin are coefficients from the general linear model, showing the simple effect of BAC for each threat type (*$p < .05$; **$p < .001$).

$t(65) = 0.49$, $p = .629$, $\eta_p^2 = .00$. In sum, the pattern of results for self-reported anxiety matched closely that for startle potentiation.

Discussion

This experiment provides clear evidence that the magnitude of alcohol SRD is greater when there is uncertainty about the severity of the upcoming threat than when the threat is well defined. This finding emerged in a task in which conditions were carefully matched for threat probability and timing (100% cue-shock pairings at 4.8 s after cue onset), amount and density of aversive stimulation (10 shocks with a 15-s mean ITI in each condition), and perceptual demands for processing threat cues (all cues were simple colored squares). The careful matching of these threat characteristics increases confidence that the differences in alcohol SRD across conditions resulted from differences in uncertainty about the intensity of the threat.

Uncertain threat intensity elicited a more robust defensive response than did comparably intense (i.e., high and low) certain threats. This finding appears to confirm the proverb, "better the devil you know than the devil you don't." It also raises the possibility that the increased alcohol SRD observed during uncertain threat resulted from the increased defensive responding in that condition rather than uncertainty per se (e.g., Moberg et al., 2011). However, this appears unlikely given that we observed comparable alcohol SRD during certain threats of high and low intensity, even though certain high threat elicited significantly greater startle potentiation than certain low threat. This significant moderation of alcohol SRD by threat uncertainty (uncertain vs. certain) but not threat intensity (certain high vs. certain low) strongly implicates threat uncertainty as the necessary characteristic for increased alcohol SRD. In other words, intoxicated drinkers may be less anxious about "the devil you don't know" than about "the devil you know," which in turn may lead to increases in certain types of risk taking when people drink (Corte & Sommers, 2005).

The current results join previous research that demonstrated an increase in alcohol SRD when participants were uncertain if and when shocks would occur (Hefner & Curtin, 2012; Hefner et al., 2013; Moberg & Curtin, 2009). Our study thus provides an important conceptual replication of these prior findings at a time when psychological science has been criticized for its inattention to the replicability of research findings (see, e.g., Pashler & Wagenmakers, 2012, a special section on replication in *Perspectives on Psychological Science*). More important, uncertainty about the nature of a threat, including its severity or intensity, appears to be qualitatively different from uncertainty about the occurrence of that threat. However, the two types of uncertainty have comparable impact on the magnitude of alcohol SRD. This suggests that threat uncertainty is a broadly relevant threat characteristic regardless of the source of the uncertainty and begins to implicate higher-level cognitive processes, including appraisal and attention that may be involved in response to uncertain threats generally (Curtin et al., 2001; Sayette, 1993). Direct measurement of these cognitive processes with varied methods (e.g., event-related potentials, startle prepulse inhibition, self-report of subjective risk and controllability) will be an important next step on the path to specifying mechanisms of action.

Our confidence in the conclusion that the magnitude of alcohol SRD is greater during uncertain than during certain threat is increased by our supplemental analyses of self-reported anxiety. This program of research on alcohol SRD during uncertain threat builds on basic affective neuroscience research with rodents (Davis et al., 2010) that relies on startle potentiation as the primary dependent measure of threat response (Hefner & Curtin, 2012; Hefner et al., 2013; Moberg & Curtin, 2009). This study is the first to include explicit measurement of self-reported anxiety during uncertain and certain threat. The finding of comparable BAC effects across both physiological and self-report measures in the same experiment

eliminates many alternative methodological or measurement explanations of the pattern of results. It also generalizes the alcohol SRD effect to the domain of subjective emotional experience and suggests that participants are aware to some degree of the greater alcohol SRD reinforcement available during uncertain threat.

Early theory and research on alcohol SRD identified alcohol dose as a potential important moderator of SRD effects, regardless of whether the threats were certain or uncertain. The influence of dose on SRD effects also has important implications for understanding how alcohol SRD will influence real-world drinking. Unfortunately, the majority of SRD research has used only a single, moderate dose of alcohol. We used a novel quantitative manipulation of BAC to probe the alcohol dose response function across a broader range of BACs. We established that alcohol SRD is linear during both certain and uncertain threat up to a moderately high BAC (0.12%). This suggests that alcohol use is negatively reinforced even from the first drink (i.e., at very low BACs) within a drinking occasion, which may indicate that preliminary, modest reinforcing effects are available to most drinkers. However, our results indicate that the magnitude of reinforcement from alcohol SRD may increase with increasing BACs. Thus, higher BACs associated with heavier, binge-drinking episodes may be more strongly reinforced despite the longer-term negative consequences associated with heavy drinking.

Our supplemental analyses of individual differences (see the Supplemental Material) indicated that the magnitude of alcohol SRD during uncertain threat was reduced among individuals who reported that they typically binge-drink alcohol outside the laboratory. Additional developmental and longitudinal research is necessary to determine if this individual difference moderator reflects a premorbid etiological risk factor for alcoholism (Schuckit & Smith, 2006; Schuckit et al., 2009) or the development of tolerance following frequent heavy use. Regardless, these drinkers may need to pursue particularly heavy, hazardous levels of alcohol to obtain rewarding alcohol SRD effects.

Alcohol SRD during uncertain threat may contribute meaningfully to the putative reward that drinkers receive from alcohol. The moderating roles of alcohol dose and binge use patterns suggest that this presumed reinforcement mechanism may encourage heavy, high-BAC, hazardous use. The alcohol SRD effects were manifest in both drinkers' defensive physiology and their subjective emotional response to uncertain threats. A next step would be to contrast these effects of alcohol with those of other relevant drugs (e.g., anxiolytics, sedatives; Grillon et al., 2006), The current findings should be directly linked to alcohol use itself via simultaneous measurement of stress and ad lib drinking in the laboratory and real world.

In real-world contexts (e.g., our opening scenario), both certain and uncertain threats are often appraised to some degree prior to drinking. Sayette (1993) has suggested that the timing of drinking relative to threat appraisal may have an important moderating effect on the magnitude of alcohol SRD. This thesis was outside the scope of our current experiment. However, future research should examine the impact of these temporal factors on alcohol SRD in the face of uncertain threats.

Compensatory neuroadaptation in the response to uncertain threats following chronic alcohol or other drug use and early chronic stress have been implicated in addiction (Koob & Volkow, 2010). In other recent research, we have provided preliminary evidence of neuroadaptation in response to uncertain threat among smokers (Hogle et al., 2010). Confirmation of a similar effect among alcoholics would implicate this mechanism in the etiology of alcoholism.

More broadly, these results join an emerging body of evidence from affective neuroscience about response to uncertain threat. Research with rodents suggests that distinct neural mechanisms are involved in response to uncertain versus certain threats (Davis et al., 2010). Clinical research implicates exaggerated response to uncertain but not certain threats in the etiology of anxiety disorders in humans (Grillon et al., 2008; Grillon, Pine, et al., 2009). The use of alcohol in the current experiment can be viewed as a coarse pharmacological manipulation that allowed us to dissociate the putatively distinct mechanisms underlying uncertain versus certain threat in humans (see Hefner et al., 2013, for additional discussion). Future neuropharmacological challenge with corticotrophin-releasing factor and norepinephrine agonists and antagonists in humans can more precisely probe these neural mechanisms that have been implicated in the response to uncertain threats (Davis et al., 2010).

Author Contributions

J. J. Curtin and D. E. Bradford developed the study concept and design. Data collection was performed by D. E. Bradford and B. L. Shapiro. D. E. Bradford performed the data analysis and interpretation under J. J. Curtin's supervision. D. E. Bradford drafted the manuscript, and J. J. Curtin provided critical revisions. All authors approved the final manuscript.

Declaration of Conflicting Interests

The authors declared that they had no conflicts of interest with respect to their authorship or the publication of this article.

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Supplemental Material

Additional supporting information may be found at http://pss.sagepub.com/content/by/supplemental-data

Notes

1. Four additional participants were regression outliers (i.e., studentized residual with Bonferroni-corrected $p < .05$) in preliminary analyses of startle potentiation. We removed these participants from the sample.
2. BAC was multiplied by 100 to increase interpretability of its GLM coefficients, such that a 1-unit increase represented a 0.01% increase in BAC. Baseline startle was mean-centered. Unit-weighted, centered orthogonal regressors were included for gender (male = 0.5, female = −0.5) and block order (Helmert coding).

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Original Article

Alcohol's effects on emotionally motivated attention, defensive reactivity and subjective anxiety during uncertain threats

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Abstract

Developing a better understanding of how and under what circumstances alcohol affects the emotions, cognitions and neural functions that precede and contribute to dangerous behaviors during intoxication may help to reduce their occurrence. Alcohol intoxication has recently been shown to reduce defensive reactivity and anxiety more during uncertain *vs* certain threat. However, alcohol's effects on emotionally motivated attention to these threats are unknown. Alcohol may disrupt both affective response to and attentional processing of uncertain threats making intoxicated individuals less able to avoid dangerous and costly behaviors. To test this possibility, we examined the effects of a broad range of blood alcohol concentrations on 96 participants' sub-cortically mediated defensive reactivity (startle potentiation), retrospective subjective anxiety (self-report) and cortically assessed emotionally motivated attention (probe P3 event related potential) while they experienced visually cued uncertain and certain location electric shock threat. As predicted, alcohol decreased defensive reactivity and subjective anxiety more during uncertain *vs* certain threat. In a novel finding, alcohol dampened emotionally motivated attention during uncertain but not certain threat. This effect appeared independent of alcohol's effects on defensive reactivity and subjective anxiety. These results suggest that alcohol intoxication dampens processing of uncertain threats while leaving processing of certain threats intact.

Key words: attention; uncertainty; stress; alcohol; threat; anxiety

Introduction

According to recent calculations, problematic alcohol use cost the United States 249 billion in 2010 alone, a cost that continues to increase (Sacks *et al.*, 2015). Dangerous and damaging behavior by a subset of intoxicated individuals makes up a large portion of this cost (e.g. medical bills from preventable injury, drunk driving, petty crime). Before we can change these behaviors, we may require better understanding of how alcohol affects the cognitive and affective processes that precede and influence them. Research into how and under what circumstances alcohol affects our emotions, attention and their neural substrates will help inform prevention and intervention efforts to decrease the risk and cost of intoxicated behavior.

An intoxicated individual may, for example, decide to walk home alone on poorly lit or otherwise unsafe streets, pursue a risky sexual encounter, or commit petty theft (e.g. shoplift)–all actions he or she might normally avoid while sober. The threats of assault or robbery, contracting a sexually transmitted disease,

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or legal consequences may generally motivate sober individuals to refrain from risky behaviors. However, these same threats appear to have less influence on intoxicated individuals for reasons that are still inadequately understood. Notably, these threats all share some degree of uncertainty. Street robberies or assaults are infrequent, symptoms of sexually transmitted diseases are often invisible, and petty theft is often not detected or punished legally. In fact, many threats in our daily life are characterized by some degree of uncertainty. In light of this, how people respond to uncertain threats in particular has recently become an important focus of cognitive, affective and neuroscience research (Davis *et al.*, 2010; Grupe and Nitschke, 2013).

Laboratory research on the neurobehavioral, cognitive and affective responses to threats distinguishes between those that are uncertain *vs* certain. These two types of threats elicit distinct patterns of innate defensive behaviors that involve activation of overlapping but separable sub-nuclei within the central extended amygdala (Davis, *et al.*, 2010). Through these varying pathways in the central amygdala, the eye blink startle response to auditory 'startle probes' is potentiated during presentation of visual cues signaling threat of both uncertain and certain shock (Davis, 2006). However, startle potentiation is selectively decreased during threat of uncertain shock by anxiolytic drugs such as benzodiazepines and alcohol (Grillon *et al.*, 2006; Kaye *et al.*, 2017). In fact, across several manipulations of threat certainty, alcohol has consistently produced a significantly greater reduction of startle potentiation when the threats were in some way uncertain, regardless of what was actually uncertain about the threats (Kaye *et al.*, 2017). Uncertain threats also elicit subjective emotional (e.g. anxious) responses (Jackson *et al.*, 2015), and alcohol, an anxiolytic drug, reduced self-reported anxious response more during uncertain than certain threats in one study (Bradford *et al.*, 2013). Furthermore, recent data suggests that alcohol is more likely to reduce negative affect in social settings when the behavior of others is uncertain (e.g. social attribution theory, Fairbairn and Sayette, 2014).

Uncertain and certain threats each may also affect attentional processes differently (Cornwell *et al.*, 2008; Blanchard *et al.*, 2011; but see Nelson *et al.*, 2015). Alcohol has been observed to impair attention in some studies and not others (see Sayette, 2017 for review), but the specific circumstances under which alcohol has these detrimental effects remains unclear (e.g. Farris *et al*, 2008). No study to our knowledge has assessed alcohol's effects on attentional processing of uncertain *vs* certain threats.

For the sober individual, threats or other emotionally relevant stimuli demand attention, a process thought to be adaptive for survival (Blanchard *et al.*, 2011; Robinson *et al.*, 2015). Researchers can unobtrusively measure this attentional demand using Event Related Potentials (ERPs). The obligatory P3 is a classic ERP usually elicited in response to unexpected or infrequent stimuli and is believed to reflect, among other things, attentional processing (see Linden, 2005 for review). However, when participants' attentional resources are engaged by an emotionally relevant foreground stimulus or context (e.g. presentation of a frightening picture or a cue that signals impending shock), the P3 to background, task-irrelevant stimuli (e.g. an occasional innocuous auditory tone) is suppressed (Schupp *et al.*, 1997; Keil *et al.*, 2007). Given putative limits on attentional resources, more attentionally engaging foreground emotional stimuli or contexts result in greater suppression of the P3 to background stimuli (Hamm *et al.*, 2007; Ferrari *et al.*, 2010).

Researchers have recently taken advantage of this suppression of the P3 as a 'relatively pure index' of emotionally motivated attention that is conveniently assessed in paradigms using startle potentiation that already include infrequent, task-irrelevant background stimuli (i.e. the auditory startle probes; Bradley *et al.*, 2006). In prior research, the P3 ERP elicited from the task irrelevant, auditory startle probes was more suppressed in sober individuals when they viewed threatening or otherwise emotionally evocative stimuli compared to when they viewed neutral stimuli (i.e. probe P3 suppression; Schupp *et al.*, 1997; Nelson *et al.*, 2015). Using these methods, researchers have recently shown increased allocation of emotionally motivated attention during presentation of visual cues signaling both uncertain and certain shock relative to during presentation of no-shock cues (Nelson *et al.*, 2015). This increased allocation of attentional resources during presentation of cues that signal both uncertain and certain threats over presentation of more benign stimuli may aid sober individuals to fully process threats and select adaptive responses in threatening situations.

Emotionally motivated attention and associated elaborative processing of uncertain threats may also be affected by alcohol. Alcohol may disrupt both affective response and attentional processing to uncertain threats making intoxicated individuals less able to avoid dangerous and costly behaviors in these situations. Because affective and attentional processes that influence behavior may not always reach conscious awareness, unobtrusive, nonconscious measure of these processes in a paradigm that allows parametric manipulation of threat certainty may be a valuable tool for understanding these processes.

Here, we report the first assessment of alcohol's effects on emotionally motivated attention during cued threat of uncertain and certain electric shocks using probe P3 suppression. We simultaneously measured startle potentiation and self-reported anxiety during these threat cues to evaluate relationships between alcohol's effects on attentional processing, defensive reactivity and subjective emotional response. This also allowed us to assess potential unique effects of alcohol on attentional processing, controlling for defensive reactivity and subjective emotional response. We assessed alcohol's effects across a broad range of blood alcohol contents (BACs) to evaluate dose response and better model real-world drinking levels (Bradford *et al.*, 2013). Based on prior research (e.g. Bradford *et al.*, 2013), we predicted alcohol would produce a greater dose-dependent reduction of startle potentiation and self-reported anxiety during uncertain compared to certain location threats. We did not offer a priori predictions about alcohol's effect on attentional processing but instead tested for this effect, both overall and when controlling for alcohol's influence on startle potentiation and self-reported anxiety.

Method

Open science

Following recommendations about research transparency (Simmons *et al.*, 2012), we have reported how we determined our sample size, all data exclusions, all manipulations and all measures in the study. Following emerging open science guidelines (Schönbrodt *et al.*, 2015), we have made the data and analysis scripts, associated with this report publicly available via Open Science Framework at osf.io/5n7hm.

Participants

The study was approved by the Institutional Review Board's Social and Behavioral Sciences human subjects committee at

the University of Wisconsin, and informed written consent was obtained from all study participants prior to their participation. We recruited 96 participants (48 female; mean age = 22.5 years, s.d. = 2.4 years) from the university community. We discarded and replaced data from an additional 6 participants before analysis due to data collection and/or equipment failure. Power analyses indicated that a sample size of 87 participants would provide 80% power to detect a medium effect size (partial eta-squared = 0.09) for the BAC X Threat type interactions for our three dependent measures, which is comparable to effect sizes seen in recent studies using similar tasks (e.g. Bradford *et al.*, 2013). We planned to recruit 96 participants to balance cell sizes across target doses, task orders, and gender. Participants were between 21 and 35 years old, had experience within the last year with drinking the amount of drinks needed to obtain the highest study dose of alcohol, reported no history of alcohol-related problems, no current psychiatric medication use, no alcohol contraindicated medical condition, were not pregnant (verified by urine sample) and were sober on arrival (verified via breathalyzer [Alcosensor IV; Intoximeters Inc., St. Louis, MO)]. We paid participants $10/h or class extra-credit points for their participation.

Baseline general startle reactivity assessment

We presented visual and auditory stimuli with a PC-based Matlab script using the Psychophysics Toolbox (Brainard, 1997; Pelli, 1997). Prior to beverage assignment, we measured participants' general startle reactivity in a shock-free baseline procedure lasting 4 min (Bradford *et al.*, 2014a; see Startle Response Measurement and Processing below). Participants viewed a series of 12 gray scale squares with no instructed meaning presented in the center of a CRT monitor for 5 s each separated by an inter-trial interval (ITI, range 10–20 s, $M = 15$ s).

Beverage manipulation

In a between-subjects manipulation, we randomly administered 4 alcohol doses (target BACs of 0.03, 0.06, 0.09 and 0.12; $N = 16$ each) and a placebo dose ($N = 32$) across the 96 participants. Alcohol doses were equally stratified by sex. Beverage manipulation methods were consistent with previous research (Bradford *et al.*, 2013; see Supplementary Materials for more information). In analyses evaluating alcohol's effects on dependent variables, we used mean achieved BAC averaged across BAC measurements for each participant taken with the breathalyzer immediately before start and after completion of the main task.

Shock procedures

After the beverage manipulation, we measured participants' subjective tolerance to a series of increasing intensity 200 ms electric shocks (7 mA maximum) using a custom shock box following standard procedures from our laboratory (Bradford *et al.*, 2014b; see Supplementary Materials for more information). We used participants' maximum tolerated shock intensity at each location (i.e. the right triceps and calf) for shocks in the main task to minimize potential effects of individual differences in subjective shock tolerance.

Cued threat of shock task

In the main task, participants viewed a series of serially presented gray-scale square visual cues presented on a CRT monitor for 5 s each and separated by an ITI (range 10–20 s, $M = 15$ s). Visual cues were presented in within-subject blocks with the same visual cue repeated three times within each block. Blocks consisted of cues that signaled impending shocks to a certain bodily location, shocks to an uncertain bodily location, or cues that signaled no-shock at all. In the center of the gray-scale square, a two-character abbreviation indicated which body location shocks would occur on (see below).

Each participant viewed four different certain location shock blocks consisting of cues signaling which of four specific bodily locations would receive an impending electric shock: the participant's right triceps (indicated with the characters 'RT'), left triceps ('LT'), right calf ('RC') and left calf ('LC'). We instructed participants that electric shocks at the indicated location would be administered at the end of the 5 s cues for all certain-location shock blocks.

Participants also viewed four uncertain location shock blocks in which all the cues included the characters '??'. We instructed participants that the location of shock would vary across these uncertain location cues within each uncertain block. In fact, all four shock locations (right triceps, left triceps, right calf and left calf) in the uncertain location blocks were equiprobable and intermixed pseudo-randomly with none of the shock locations repeated more than once within a block. In all shock blocks, 200 ms shocks were administered at 4.8 s post-cue onset.

Finally, participants viewed four no-shock blocks (including the characters 'NS'). We instructed participants that no shocks would be administered in no-shock blocks and during ITIs in any block. A pre-block message on the monitor indicated the start of each block type. Blocks were presented in one of eight pseudo-random orders with half starting with an uncertain block and half starting with each of the four certain blocks, counterbalanced across participants.

After the main task and BAC assessment, participants rated how anxious they were when they saw each cue type, using a 7 point rating scale (1 = 'not at all anxious'; 7 = 'extremely anxious'). Participants then completed a placebo manipulation check, a battery of self-report individual difference questionnaires for goals not relevant to the current student (see Supplemental Materials), were debriefed, compensated and dismissed once reaching a BAC below 0.03%.

Startle response measurement and processing

A Neuroscan Synamps bioamplifier (Compumedics Neuroscan, Charlotte, NC) sampled the electromyographic signal at 2500 Hz from two 4 mm Ag-AgCl sensors (TDE-023; Discount Disposables, St. Albans, VT) filled with conductive gel (ECI Electro-Gel; Electrocap International, Eaton, OH) placed over the orbicularis oculi muscle under the right eye according to published guidelines (Blumenthal *et al.*, 2005; Bradford *et al.*, 2014b).

We measured the eye-blink startle response to acoustic startle probes (50 ms, 102 dB white noise with near instantaneous rise time). We presented 6 noise probes during a subset of the visual cues at 3.5 or 4.5 s post cue onset with equal probable timing during the baseline procedure. We presented 24 noise probes during a subset of the cues in the threat of shock (16 probes; 8 for each threat type) and no-shock (8 probes) blocks at 3.5 or 4.5 s, post cue onset, with equal probable timing during the main task (see Supplementary Materials for more information).

We conducted offline data processing for all physiological signals using the PhysBox plugin (Curtin, 2011) within the EEGLab toolbox (Delorme and Makeig, 2004) in MATLAB (*MATLAB and Statistics Toolbox*, 2013). We followed published

guidelines for startle response reduction and processing (Blumenthal *et al.*, 2005; Bradford *et al.*, 2014b). We excluded from all analyses one participant, whose mean baseline general startle reactivity was <5 microvolts (i.e. non-responder).

Probe P3 measurement and processing

The Neuroscan bioamplifier also sampled the EEG signal at 2500 Hz from nine scalp sites (Fz, F3, F4, Cz, C3, C4, Pz, P3, P4) with conductive gel in a custom Electro-cap (ECI Electro-Gel, Electro-Cap International; Eaton, OH). We referenced signals online to the left mastoid and re-referenced offline to averaged mastoids. We measured vertical electrooculogram (VEOG) activity to correct for eyeblink artifact. We measured the P3 ERP to the acoustic startle probes at electrode site Pz, consistent with most published reports (e.g. Alius *et al*, 2015; Nelson and Hajcak, 2017). Data from the other electrode sites were collected for goals not relevant to the current study (reported in the Supplementary Materials).

EEG response reduction and processing followed published guidelines (Picton *et al.*, 2000). We removed three participants from ERP analyses due to excessive noise in the VEOG data that prevented use of eyeblink artifact correction. We removed one participant with >25% of trials rejected as EEG artifact from ERP analysis. We selected the scoring window of 250–350 ms post probe to be consistent with previous research (e.g. Benning *et al.*, 2015; see Supplementary Materials for further information). We scored the probe P3 as mean response in this window in each condition (Figure 1).

Analysis plan

We calculated general startle reactivity as mean startle response to startle probes during cues in the baseline procedure. We calculated startle potentiation as the increase in startle response to probes during cues in the shock blocks relative to cues in the no-shock blocks, self-reported anxiety as the increase in anxiety to the shock cues relative to the no-shock cues and probe P3 suppression as the decrease in the P3 ERP to probes during cues in the shock blocks relative to cues in the no-shock blocks.

We analyzed startle potentiation, self-reported anxiety, and probe P3 suppression with threat type (mean of response across all uncertain cues–mean of response across all certain cues) as a within-subjects factor in separate, fully interactive, General Linear Models (GLMs) using R Studio (*RStudio: Integrated development environment for R*, 2016) for R (R Development Core Team, 2015) with the lmSupport (Curtin, 2015) package. All GLMs included quantitative, between-subjects regressors for BAC and general startle reactivity (mean centered). All GLMs also included task block order using unit weighted, centered, orthogonal regressors. We report both partial eta-squared (η_p^2) and GLM coefficients (b) to describe effect sizes. We conducted outlier analysis (studentized residual with Bonferroni corrected $P < 0.05$) in preliminary GLMs for each dependent variable. This resulted in the removal of one outlier each for GLMs involving startle potentiation and self-reported anxiety.

We conducted supplemental analyses to clarify the shape of the alcohol dose response function by adding a regressor to rule out a quadratic BAC effect to each of the GLMs described above (Bradford *et al.*, 2013). We assessed if the BAC X threat type

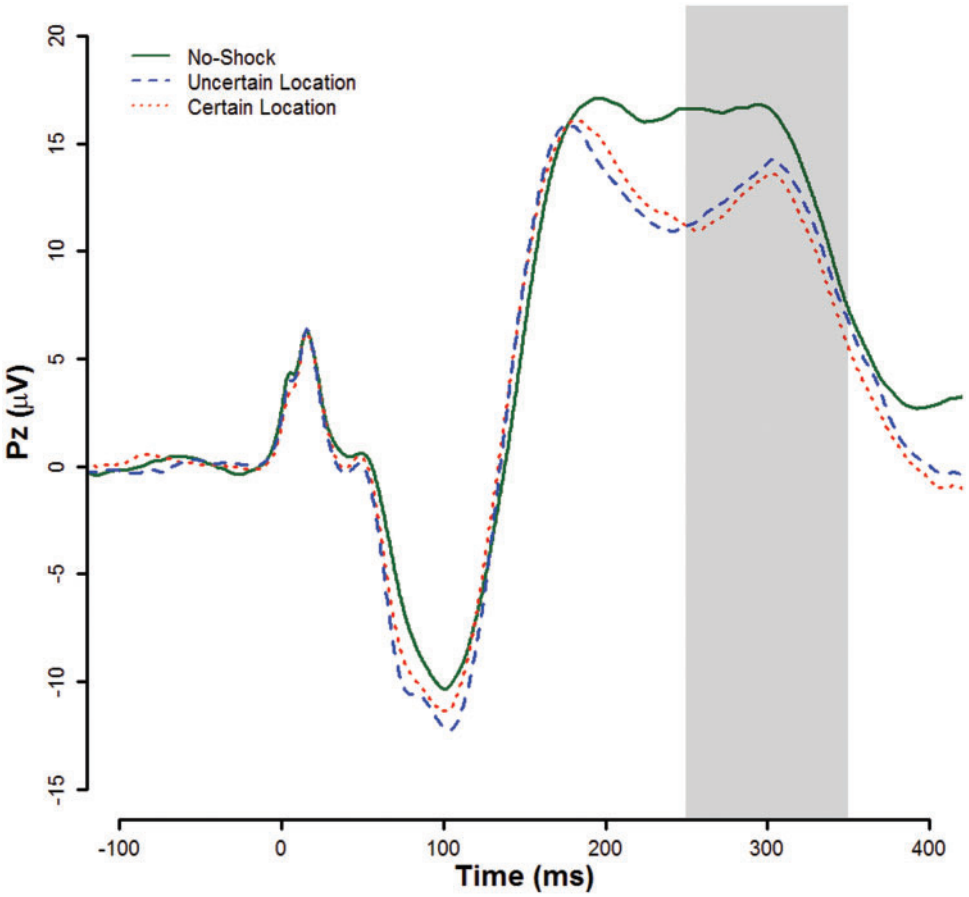

Fig. 1. Grand average event related potentials to the auditory startle probe by threat type. Gray band indicates scoring window for probe P3.

interaction for probe P3 suppression was independent from alcohol's effects on startle potentiation and self-reported anxiety by testing this interaction controlling for startle potentiation and self-reported anxiety (uncertain–certain) difference scores by including each of these scores additively in separate GLMs otherwise identical to the primary GLM for probe P3. Finally, we tested the bivariate correlations between the uncertain - certain difference scores for all DVs to test for further evidence that each DV contrast reflected distinct processes.

Results

BAC

The mean BAC for participants administered alcohol was 0.048% (s.d. = 0.04%) immediately before the main task, and 0.048% (s.d. = 0.05%) immediately after the task. The placebo manipulation was successful in establishing expectations of alcohol consumption and intoxication across BACs (see Supplementary Materials for full analysis of the placebo manipulation). Observed BAC was multiplied by 100 in the following analysis to increase interpretability of its GLM coefficients, such that a 1-unit increase represented a 0.01% increase in BAC.

Startle potentiation

Spearman-Brown corrected split half (odd *vs* even trials) internal consistency for startle magnitude was: no-shock $r_{sb} = 0.96$, certain-threat $r_{sb} = 0.97$ and uncertain-threat $r_{sb} = 0.96$ and startle potentiation was: certain-threat $r_{sb} = 0.64$ and uncertain-threat $r_{sb} = 0.65$.

At a BAC of 0.00%, mean startle potentiation was significant (nonzero) overall, $\eta_p^2 = 0.36$, $b = 27.2$, $t(86) = 6.97$, $P < 0.001$ and separately during uncertain ($\eta_p^2 = 0.38$, $b = 33.1$, $t(86) = 7.24$, $P < 0.001$) and certain ($\eta_p^2 = 0.27$, $b = 21.2$, $t(86) = 5.71$, $P < 0.001$) threats (Figure 2). Startle potentiation was significantly greater during uncertain threats than certain threats, $\eta_p^2 = 0.16$, $b = 11.9$, $t(86) = 4.04$, $P < 0.001$.

As predicted, the BAC X threat type interaction was significant, $\eta_p^2 = 0.05$, $b = -1.0$, $t(86) = 2.11$, $P = 0.038$, such that the BAC effect on startle potentiation was significantly greater during uncertain ($\eta_p^2 = 0.05$, $b = -1.6$, $t(86) = 2.22$, $P = 0.029$) than certain threats ($\eta_p^2 = 0.01$, $b = -0.6$, $t(86) = 1.06$, $P = 0.292$). The quadratic BAC X threat type effect was not significant, $\eta_p^2 = 0.01$, $b = 0.1$, $t(85) = 0.86$, $P = 0.390$.

Self-reported anxiety

At a BAC of 0.00%, mean retrospective self-reported anxiety was significant (nonzero) overall, $\eta_p^2 = 0.78$, $b = 3.5$, $t(86) = 17.32$, $P < 0.001$ and separately during uncertain ($\eta_p^2 = 0.81$, $b = 4.3$, $t(86) = 19.21$, $P < 0.001$) and certain ($\eta_p^2 = 0.68$, $b = 2.7$, $t(86) = 13.59$, $P < 0.001$) threats (Figure 3). Self-reported anxiety was significantly greater during uncertain threats than certain threats, $\eta_p^2 = 0.65$, $b = 1.6$, $t(86) = 12.66$, $P < 0.001$.

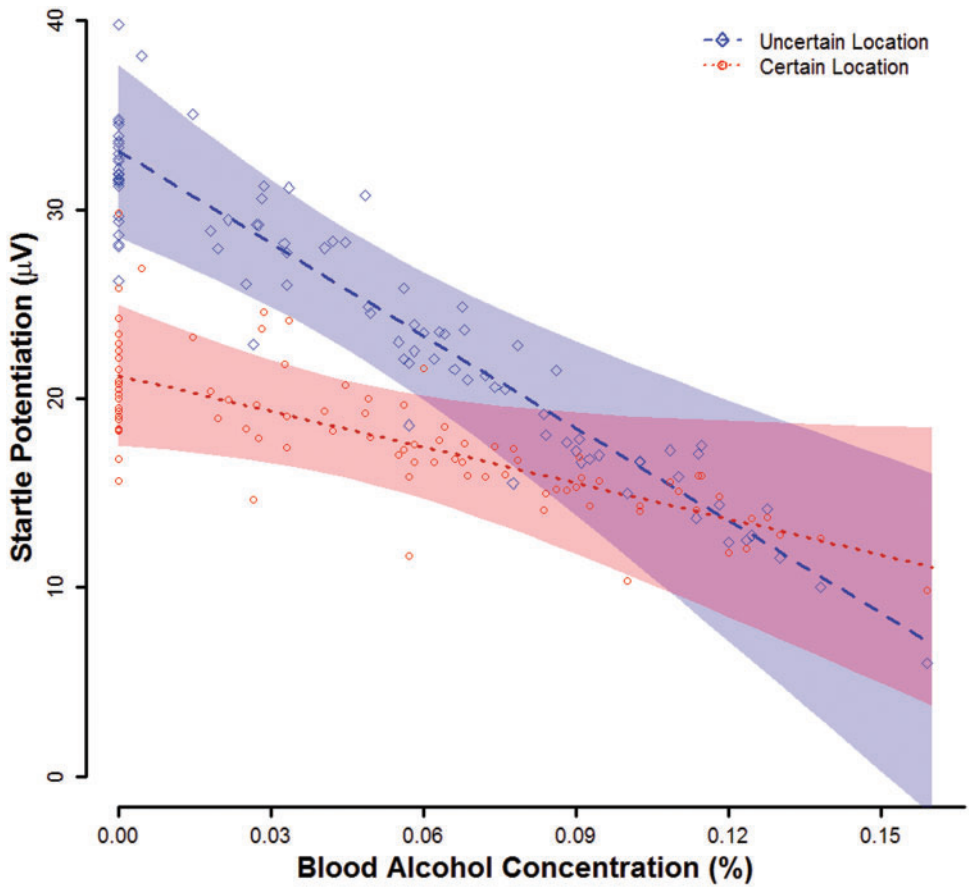

Fig. 2. Startle potentiation by BAC and Threat Type. Lines display point estimates for mean startle potentiation by BAC and threat type from the general linear model. Translucent bands indicate confidence envelopes (±1 SE) for these point estimates. Points represent participants' startle potentiation residual scores relative to their predicted values and scaled by the square root of N to allow display on the same scale as the population mean point estimates.

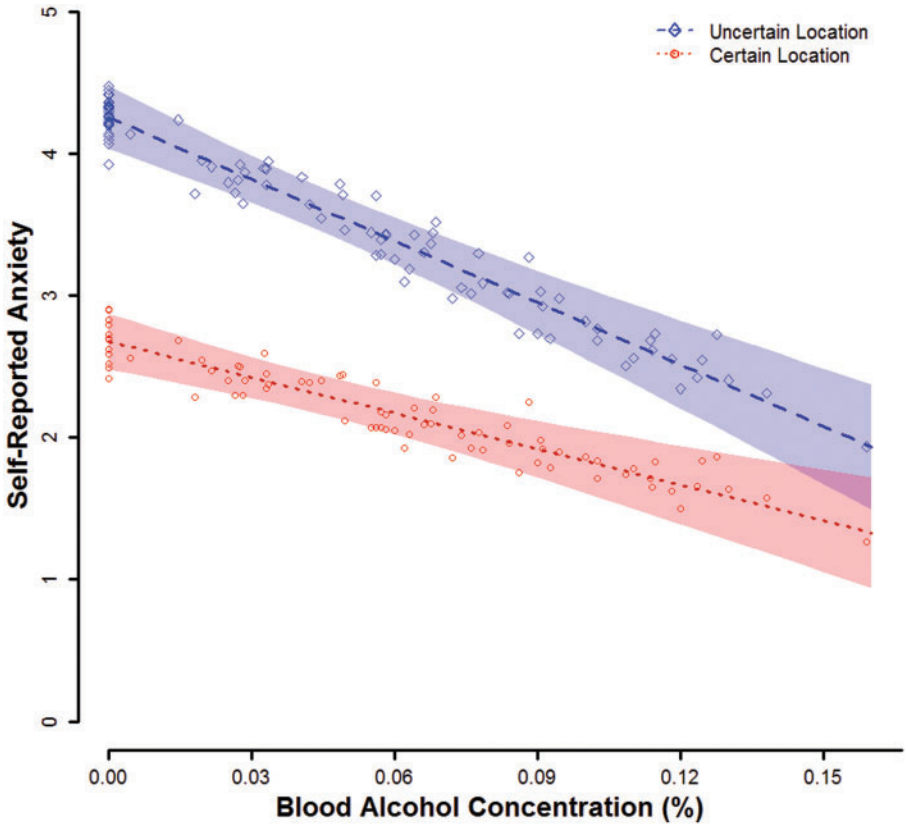

Fig. 3. Self-reported anxiety by mean BAC and threat type. Lines display point estimates for mean self-reported anxiety by BAC and threat type from the general linear model. Translucent bands indicate confidence envelopes (±1 SE) for these point estimates. Points represent participants' self-reported anxiety residual scores relative to their predicted values and scaled by the square root of N to allow display on the same scale as the population mean point estimates. Figure © 2017 John Curtin, Daniel Bradford, Courtney Motschman, and Mark Starr under Creative Commons Attribution 4.0 International Public License CC-By.

As predicted, the BAC X threat type interaction was significant, $\eta_p^2 = 0.10$, $b = -0.1$, $t(86) = 3.04$, $P = 0.003$, such that the BAC effect on self-reported anxiety was significantly greater during uncertain ($\eta_p^2 = 0.16$, $b = -0.1$, $t(86) = 4.08$, $P < 0.001$) than certain threats ($\eta_p^2 = 0.08$, $b = -0.1$, $t(86) = 2.66$, $P = 0.009$). The quadratic BAC X threat type effect was not significant, $\eta_p^2 < 0.01$, $b = 0.0$, $t(85) = 0.62$, $P = 0.536$.

Probe P3 suppression

Spearman-Brown corrected split half (odd *vs* even trials) internal consistency for probe P3 magnitude was: no-shock $r_{sb} = 0.79$, certain-threat $r_{sb} = 0.63$ and uncertain-threat $r_{sb} = 0.73$ and probe P3 suppression was: certain-threat $r_{sb} = 0.34$, and uncertain-threat $r_{sb} = 0.47$.

At a BAC of 0.00%, mean startle probe P3 suppression was significant (nonzero) overall, $\eta_p^2 = 0.17$, $b = 3.8$, $t(83) = 4.06$, $P < 0.001$ and separately for uncertain ($\eta_p^2 = 0.16$, $b = 4.3$, $t(83) = 4.03$, $P < 0.001$) and certain ($\eta_p^2 = 0.11$, $b = 3.3$, $t(83) = 3.28$, $P = 0.002$) threats (Figure 4). Probe P3 suppression was comparable during uncertain threats and certain threats, $\eta_p^2 = 0.02$, $b = 1.04$, $t(83) = 1.17$, $P = 0.244$.

The BAC X threat type interaction was significant, $\eta_p^2 = 0.08$, $b = -0.4$, $t(83) = 2.73$, $P = 0.008$, such that the BAC effect on probe P3 suppression was significantly greater during uncertain ($\eta_p^2 = 0.07$, $b = -0.4$, $t(83) = 2.44$, $P = 0.017$) than certain threats ($\eta_p^2 < 0.01$, $b = 0.0$, $t(83) = 0.18$, $P = 0.855$). The quadratic BAC X threat type effect was not significant, $\eta_p^2 = 0.02$, $b = 0.0$, $t(82) = 1.16$, $P = 0.251$. The BAC X threat type interaction for probe P3 suppression remained significant in GLMs that controlled for individual differences in either startle potentiation ($\eta_p^2 = 0.09$, $b = -0.4$, $t(82) = 2.80$, $P = 0.006$) or self-reported anxiety ($\eta_p^2 = 0.10$, $b = -0.4$, $t(82) = 2.94$, $P = 0.004$). No significant correlations were observed among the uncertain–certain difference scores for any of these three measures, probe P3 and startle potentiation ($r = -0.01$, $P = 0.942$), probe P3 and self-reported anxiety ($r = 0.7$, $P = 0.487$), and startle potentiation and self-reported anxiety ($r = 0.13$, $P = 0.229$). Post-hoc, exploratory analysis suggested the relationships among these dependent variables did not change across the varying BACs (see Supplementary Materials).

Discussion

Cues for both uncertain and certain threats elicited robust negative affective response (i.e. defensive reactivity, subjective anxious response) and increased attention among sober participants as indexed by startle potentiation, self-report and Probe P3, respectively. However, uncertain threats increased defensive reactivity and subjective anxiety more potently than certain threats. These observations join recent experimental and other evidence (Koolhaas *et al.*, 2011; Bradford *et al.*, 2013; Jackson *et al.*, 2015) to indicate that uncertain threats are generally more affectively aversive and/or anxiogenic than certain threats. Nonetheless, it appears that cues for uncertain and certain threat recruited comparable attention resources as indicated by comparable suppression of attentional response to the startle probe during each threat cue relative to the no-shock cues (Nelson *et al.*, 2015). In other words, even though uncertain threats prompt stronger

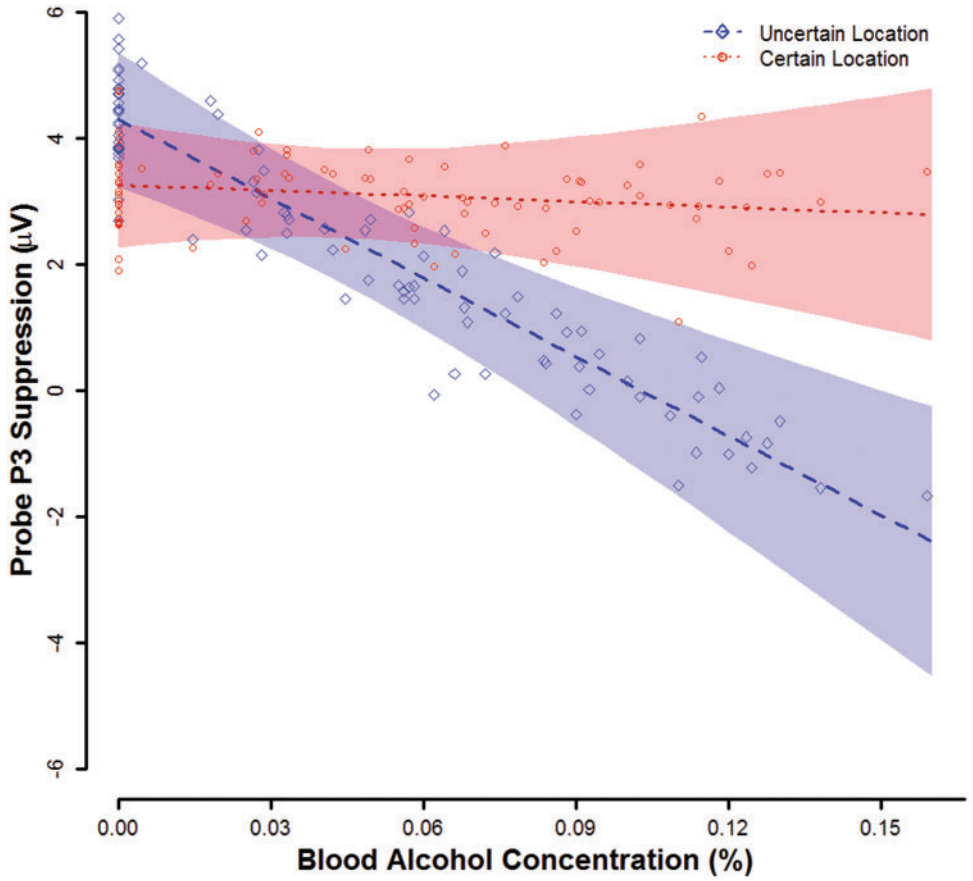

Fig. 4. Probe P3 Suppression by Mean BAC and Threat Type. Lines display point estimates for mean probe P3 suppression by BAC and threat type from the general linear model. Translucent bands indicate confidence envelopes (±1 SE) for these point estimates. Points represent participants' probe P3 suppression residual scores relative to their predicted values and scaled by the square root of N to allow display on the same scale as the population mean point estimates.

negative affective response, all threats appear to increase attentional processing that may be critical to support adequate appraisal and subsequent adaptive behavioral response, at least among sober individuals. These data also highlight that the stimulus characteristics and neurobiological systems that drive affective response are at least partially separable from the characteristics that recruit increased attention (Berridge *et al.*, 2009).

Decades of research broadly indicate that the affective 'stress response dampening (SRD)' properties of alcohol reinforce alcohol use among both recreational drinkers and alcoholics alike (Sher, 1987; Sayette, 2017). However, it remains important to clarify when, how and for whom alcohol SRD occurs to answer fundamental questions about this popular drug's reinforcing effects and to mitigate the negative consequences associated with its excessive use (Bradford *et al.*, 2013). We have now generated robust evidence that alcohol SRD is most potent 'when' the threats are characterized by some degree of uncertainty, regardless if this uncertainty concerns the threat's location (this study), probability (Hefner and Curtin, 2012), timing (Hefner *et al.*, 2013), combination of probability and timing (Moberg and Curtin, 2009), or intensity (Bradford *et al.*, 2013). Thus, we have begun to speculate 'how' alcohol provides SRD, implicating neural mechanisms involving CRF and NE sensitive pathways in the central extended amygdala that selectively mediate startle potentiation to uncertain threats (Davis *et al.*, 2010). Recently emerging theory and empirical evidence regarding 'who' may be most sensitive to alcohol SRD implicates the role of strong negative affective reinforcement among alcoholics with a history of chronic, heavy alcohol use (Kaye *et al.*, 2017; Moberg *et al.*, 2017).

To our knowledge, the current study provides the first evidence that alcohol dose-dependently disrupts attention during uncertain threats but not certain threats as indicated by decreased probe P3 suppression during uncertain but not certain threats. Equally important, this selective disruption in attentional processing appears to be independent of alcohol's effects on negative affect. As such, the effect of alcohol on attention to uncertain threats may offer an additional source of reinforcement for drinking. Uncertain threats (e.g. academic or professional performance reviews, novel social interactions, many financial stressors) may make us anxious, but they can also occupy our limited attentional resources. Such perseverative rumination on these uncertain threats may interfere with adaptive flexible allocation of attention to other pressing decisions, tasks, or behaviors. To the degree that alcohol may interrupt this focus and release these attentional resources for other uses, 'drinking to forget' may be reinforcing and even adaptive in some select situations.

As we have described, alcohol's effect on attention and negative affective response to uncertain threats may be reinforcing. However, these same two effects may also each contribute to maladaptive decision-making and/or risky behavior during uncertain threats. Clearly, adequate attention to and appraisal of threats are fundamental to good decision-making

(Fernandes *et al.*, 2013). Similarly, affect plays an important role in guiding adaptive behavior (Charpentier *et al.*, 2016). The current study suggests that alcohol independently impairs both of these important decision-making processes when intoxicated individuals are presented with uncertain threats. For example, the inebriated drinker who considers driving home from the bar may not adequately fear or attend to the potential but uncertain negative consequences (arrest, injury to self or others). They may also not fully attend to and appraise their own level of intoxication or alternative options available to them to get home (e.g. government sponsored 'safe-ride' programs, public transportation, sober friends). Viewed through this lens, the decision to drive home drunk may seem less surprising though still clearly costly to both the drinker and society. These impairments in attention and/or negative affective response may individually or together with other processes (e.g. reduced inhibitory control; Weafer *et al.*, 2014), help to explain many maladaptive or otherwise risky intoxicated behaviors observed under uncertain threats (e.g. sexual risk-taking, certain types of aggressive behavior, petty theft, excessive gambling at casinos). On the other hand, the current results suggest that attention and affective response to certain threats are somewhat immune to alcohol. Thus, intoxicated behavior may seem less inappropriate when the threats are imminent and highly probable (e.g. the respectful intoxicated driver cooperating with the police officer after being pulled over). Furthermore, education, law enforcement and public policy efforts that shift the balance of consequences from uncertain to more certain may also yield benefits to both drinkers and their community.

This study reinforces the importance of recent calls 'to continue to develop methods for assessing cognitive and affective processes simultaneously' in human drug research (Sayette, 2017). Many existing theoretical perspectives focus on alcohol's effects on both affective and cognitive processes including attention, working memory and appraisal to account for its reinforcing effects, its impact on behavior and etiological mechanisms for alcohol use disorder (see Sayette, 2017 for review). However, these perspectives differ with respect to the presumed causal connections and ordering among these processes. Concurrent measurement of self-reported affect, startle potentiation, and probe P3 suppression in this study allowed us to conclude that alcohol has independent effects on negative affect and attention during presentation of threats rather than alterative mediated pathways that were possible but not consistent with our data. Yet other work using simultaneous measurement of cognitive and affective processing suggests alcohol's effects on emotional responses may be mediated by other types of attentional processing (e.g. explicit focus of attention in complex visual environments; Curtin *et al.*, 2001). Future research can strengthen conclusions about the independence or causal inter-connections among various affective and cognitive processes by including direct manipulations of attention, appraisal and working memory as well (e.g. Casbon *et al.*, 2003). We also believe that future research on the cognitive-attentional effects of alcohol should continue to examine these processes in the context of affective or otherwise motivationally relevant stimuli as we do in this study. Previous research has often explored alcohol's effects in traditional cognitive tasks (e.g. Stroop, Curtin and Fairchild, 2003; N-back, Casbon *et al.*, 2003). While informative, alcohol's most salient effects on decision-making and behavior occur in affectively-charged contexts.

In this study, we demonstrated that alcohol dose-dependently reduced negative affective response and disrupted attentional processing during threats by concurrent measurement of subjective anxious response, defensive reactivity and probe P3 suppression. Alcohol's effects on affect and attention were limited to threats that were uncertain, with response during certain threats spared even at relatively high levels of intoxication. Furthermore, the effects of alcohol on negative affective response and attention appeared to occur via independent pathways. As such, we have identified two separate mechanisms that may both reinforce alcohol use but also account for maladaptive behavior in some situations.

Several aspects of this work deserve further scrutiny in future research. First, we did not explicitly measure volitional behavior in the current study. While affect and attentional processes surely influence behavior, future work can combine our methods with paradigms from behavioral economics and related disciplines to directly test the causal impact of these impairments on decision-making and ultimately behavior. The use of measures such as eye tracking or imbedded reaction time tasks may further clarify, in real time, participants' explicit focus of attention during these tasks. While subcortical CRF and NE sensitive pathways in the central extended amygdala are implicated in alcohol's selective dampening of uncertain startle potentiation, the neural mechanisms responsible for alcohol's effects on cortical structures associated with probe P3 and other measures of attention are less well understood. Furthermore, previous work suggests that alcohol's effects on uncertain startle potentiation are pharmacological in nature. Our use of a placebo manipulation but no 'true' no-alcohol condition did not allow full assessment of the relative contribution of potential expectancy and pharmacological effects that may have influenced attentional processing in the current study. Nonetheless, future research can clarify the neural circuits and neurotransmitter systems implicated in this study by more direct manipulation (e.g. administration of neurotransmitter agonists and antagonists, transcranial magnetic stimulation) or measurement (neuroimaging techniques including MEG, fMRI, PET).

This study extends other recent research to suggest that uncertainty, broadly defined, is an important characteristic of threats that modulates the degree of impairment produced by alcohol intoxication. Future research can examine these effects in more ecologically valid and socially relevant contexts such as in the presence of drinking partners and other social interactions (e.g. Fairbairn and Sayette, 2014; Sayette, 2017). In addition, other potentially important characteristics of threats such as their intensity and controllability should also be carefully considered in future research. Furthermore, we did not assess alcohol's well documented effects on responses to appetitive stimuli (e.g. Berridge *et al.*, 2009). It remains to be seen if alcohol's interactions with uncertainty extend to responses to uncertain rewards or other appetitive stimuli. Research using tasks similar to the current study but with uncertain rewards is an important next step to fully characterize alcohol's broad effects. Finally, the impact of policy changes targeting uncertainty reduction to reduce the societal costs of intoxicated behavior could be examined in the laboratory and field to evaluate policy effectiveness but also as further test of the influence of these mechanisms on behavior.

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supervision. D. E. Bradford drafted the manuscript, and all other authors provided critical revisions. All authors approved the final manuscript.

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Supplementary data

Supplementary data are available at *SCAN* online.

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Increased Startle Potentiation to Unpredictable Stressors in Alcohol Dependence: Possible Stress Neuroadaptation in Humans

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Stress plays a key role in addiction etiology and relapse. Rodent models posit that following repeated periods of alcohol and other drug intoxication, compensatory allostatic changes occur in the central nervous system (CNS) circuits involved in behavioral and emotional response to stressors. We examine a predicted manifestation of this neuroadaptation in recently abstinent alcohol-dependent humans. Participants completed a translational laboratory task that uses startle potentiation to unpredictable (vs. predictable) stressors implicated in the putative CNS mechanisms that mediate this neuroadaptation. Alcohol-dependent participants displayed significantly greater startle potentiation to unpredictable than predictable stressors relative to nonalcoholic controls. The size of this effect covaried with alcohol-related problems and degree of withdrawal syndrome. This supports the rodent model thesis of a sensitized stress response in abstinent alcoholics. However, this effect could also represent premorbid risk or mark more severe and/or comorbid psychopathology. Regardless, pharmacotherapy and psychological interventions may target unpredictable stressor response to reduce stress-induced relapse.

General Scientific Summary

Stress plays a key role in addiction etiology and relapse, but the understanding of specific mechanisms for these relationships remain limited. Rodent models suggest that repeated alcohol use changes the central nervous system circuits involved in behavioral and emotional response to stressors. This study provides preliminary support that indicates similar changes may occur from alcohol use by human alcoholics such that they experience an exaggerated response to unpredictable stressors when abstinent.

Keywords: addictive disorders, affective neuroscience, psychopharmacology, stress, substance disorders

Stress plays a key role in addiction etiology and relapse, but the understanding of the specific mechanisms remains limited (Kaye, Bradford, Magruder, & Curtin, in press). Behavioral neuroscience research in rodents has provided strong evidence to document the role of stress in alcohol and other drug (AOD) addiction (Koob & Le Moal, 2008a). Chronic AOD use in rodents causes heightened anxiety-like behavioral responses to stressors during periods of AOD deprivation (George et al., 2007; Olive, Koenig, Nannini, & Hodge, 2002). Stressors also potently instigate relapse in rodents (i.e., stress-induced reinstatement; Mantsch, Baker, Funk, Lê, & Shaham, 2016). These stress-induced behaviors are largely dependent on central nervous system (CNS) mechanisms involving corticotropin-releasing factor (CRF) and norepinephrine (NE), among other neurotransmitters, in the central extended amygdala (Koob & Le Moal, 2008a). Rodent models posit that repeated homeostatic adjustments in brain stress systems to acute periods of AOD intoxication eventually lead to long-lasting, compensatory allostatic changes in the structures and circuits involved in behavioral and emotional response to stressors (i.e., stress neuroadaptations; Koob & Le Moal, 2008a).

In humans, these stress neuroadaptations are hypothesized to result in dysregulated emotional response to stressors on cessation of use and provide the strong motivational press for further use that manifests as craving and increased risk for relapse when stressed (Baker, Piper, McCarthy, Majeskie, & Fiore, 2004; Koob & Le Moal, 2008a). AOD-dependent individuals report elevated negative affect (e.g., anxiety) when abstinent, particularly in response

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to stressors (H. C. Fox, Bergquist, Hong, & Sinha, 2007; McKee et al., 2011) and at increasing levels before AOD lapses during quit attempts (Berkman, Dickenson, Falk, & Lieberman, 2011; Kenford et al., 2002). Furthermore, laboratory stressor-induced craving has been shown to predict shorter time to relapse among AOD patients (Higley et al., 2011; Sinha, Garcia, Paliwal, Kreek, & Rounsaville, 2006). The majority of biological studies of the stress response in human AOD samples have focused on measures of hypothalamic-pituitary-adrenocortical-axis peripheral nervous system functioning (e.g., salivary cortisol; al'Absi, 2006; Sinha, 2008). However, rodent models clearly implicate neuroadaptations in extrahypothalamic CRF/NE circuits as a critical mechanism for sensitized stressor-induced behaviors in addiction. Human addiction research has not sufficiently focused on these CNS mechanisms to date. Moreover, cross-species "bench-to-bedside" research is common but often done with methods that are so divergent across species that much gets lost in translation.

Startle potentiation provides a noninvasive, psychophysiological index of heightened defensive response to stressors. It has been employed with rodents, nonhuman primates, and humans using highly parallel methods and measures, positioning it as an attractive, truly translational measure (Davis, Walker, Miles, & Grillon, 2010; Grillon & Baas, 2003). Rodent models of startle potentiation measured specifically during *unpredictable* stressors have confirmed involvement of NE and CRF sensitive pathways through the lateral divisions of the central amygdala and bed nucleus of the stria terminalis (BNST; Davis et al., 2010). These are the same CNS circuits that show sensitized stress neuroadaptations following chronic AOD use and mediate stress-induced relapse in rodents. In this study, we focus on the contrast of startle potentiation during unpredictable versus predictable stressors to test predictions from rodent models about CNS stress neuroadaptations in human alcoholics. This explicit focus on the unpredictable (vs. predictable) startle potentiation contrast uses the predictable condition to control for overall differences in defensive reactivity across stressors to allow more precise targeting of mechanisms selectively recruited by unpredictable stressors (Davis et al., 2010). Such control is particularly important to evaluate group differences when groups are not randomly assigned (e.g., alcoholics vs. healthy controls). Startle potentiation during unpredictable (vs. predictable) stressors has proven sensitive to the stress response–dampening effects of alcohol in previous related research (e.g., Bradford, Shapiro, & Curtin, 2013; Moberg & Curtin, 2009).

We predicted that alcoholics in early protracted abstinence would display sensitized response to unpredictable stressors, manifest as selectively elevated startle potentiation to unpredictable (vs. predictable) stressors. This prediction in humans rests on the substantial evidence base from rodent models reviewed here and elsewhere (Kaye et al., in press; Koob & Le Moal, 2008a). Our observations that a single acute administration of alcohol selectively reduces human startle potentiation to unpredictable (vs. predictable) stressors provides evidence that alcohol may impact these stress mechanisms and provide a press for compensatory neuroadaptation among alcoholics following chronic heavy use (Bradford et al., 2013; Hefner & Curtin, 2012; Hefner, Moberg, Hachiya, & Curtin, 2013; Moberg & Curtin, 2009). Furthermore, we have previously demonstrated that 24-hr nicotine-deprived smokers display increased response to unpredictable stressors (Hogle, Kaye, & Curtin, 2010).

We also examined four focal individual differences to guide future research into the potential causes, correlates, and consequences of the predicted sensitized response to unpredictable stressors in alcoholics. Specifically, we tested for covariation across alcoholics in the size of their unpredictable (vs. predictable) startle potentiation and (a) *alcohol-related problems,* to document the clinical consequences; (b) *presence of a withdrawal syndrome,* to establish a clinical symptom correlate of this effect; (c) *duration of abstinence,* to evaluate the stability of this effect across alcoholics at different points in their recovery, and (d) *quantity of alcohol use,* to begin to examine potential causes of the effect.

Method

Following recommendations about research transparency (Simmons, Nelson, & Simonsohn, 2012), we have reported how we determined our sample size, all data exclusions, all manipulations, and all measures in the study. Following emerging open science guidelines (Schönbrodt, Maier, Heene, & Zehetleitner, 2015), we have made the data, analysis scripts, questionnaires, and other study materials associated with this report publicly available via Open Science Framework. These materials can be accessed at https://osf.io/ykmuh. In addition, recent high-profile articles have highlighted concerns about the robustness and replicability of scientific research (Ioannidis, 2005; Open Science Collaboration, 2015; Simmons, Nelson, & Simonsohn, 2011). In particular, excessive researcher degrees of freedom have been targeted as one important contributor to these problems. To reduce concern about researcher degrees of freedom impacts on our primary results, we report robustness analyses. This allows for increased confidence that conclusions about our primary results are not dependent on selection of one specific analytic strategy.

Participants

We recruited 115 participants (58 alcoholics and 57 nonalcoholic controls) via flyers, online advertisements, and word of mouth. We required those in the *alcoholic* group to meet criteria for alcohol dependence according to the *Diagnostic and Statistical Manual of Mental Disorders* (4th ed., text rev.; *DSM–IV–TR;* American Psychiatric Association, 2000). Alcoholics also had to self-report abstinence from alcohol for a minimum of 1 week but no more than 2 months at the time of their experimental session. We required participants in the nonalcoholic control group to report no lifetime history of alcohol dependence or current alcohol abuse. We excluded participants from both groups if they reported lifetime history of illicit substance dependence, lifetime history of any severe and persistent mental illness (e.g., bipolar disorder, schizophrenia, or other psychotic disorders), current use of any medication known to affect the startle response, or any medical condition that contraindicated their safe participation. We compensated participants with $25 per hour.

We determined the sample size for this experiment to provide adequate power to test the critical contrast between alcoholic versus control participants for unpredictable versus predictable startle potentiation. Specifically, we selected a target sample size of 128 participants (64 per group) to provide 80% power to detect a moderate effect size ($d = .5$) increase in unpredictable (vs.

predictable) startle potentiation among alcoholics relative to controls using a two-tailed alpha of .05 (Cohen, 1992). We terminated data collection early when we reached a sample size of 115 participants as a result of a slower recruitment rate than anticipated and a deadline for project completion for the lead author's dissertation requirement.

Procedure

All procedures were approved by the Social and Behavioral Sciences Institutional Review Board at the University of Wisconsin—Madison. We determined preliminary eligibility during a phone screening. At a subsequent in-person screening session, participants provided informed consent after receiving information about study procedures and protections provided by the National Institutes of Health's Certificate of Confidentiality. A clinician conducted a Timeline Follow-Back (Sobell & Sobell, 1992) to assess alcohol use (last 28 days for control participants; last 28 days prior to their most recent cessation of use for alcoholics). Finally, the clinician conducted the Structured Clinical Interview for DSM Disorders—Research Version (SCID–RV; First, Spitzer, Gibbon, & Williams, 2002) to assess for *DSM–IV–TR* alcohol use disorders and other psychiatric conditions relevant to inclusion–exclusion criteria. Eligible participants were scheduled to return for an experimental session.

At the experimental session, participants' blood alcohol concentration (BAC) was assessed via breath test to confirm a BAC of .00% as required for participation. Alcoholics also reported their baseline alcohol craving (Love, James, & Willner, 1998), which was used to determine a safe level of craving for end-of-session release.

We next assessed participants' startle reactivity during a series of 12 cues (i.e., colored squares) presented on a computer monitor. Each cue was presented for 5 s with a variable intertrial interval (ITI; range = 15–20 s). Eight startle-eliciting acoustic probes were presented during the cues. An additional two probes were presented during the ITIs to reduce probe predictability, and three probes were presented at the start of this procedure to habituate the nonlinear portion of the startle response. Startle reactivity was calculated as the mean startle magnitude to the eight probes presented during the cues (see the Startle response section for additional detail). Startle reactivity was included in all analyses of startle potentiation to increase power to test predicted effects as recommended by Bradford, Kaye, and Curtin (2014; see also Bradford, Starr, Shackman, & Curtin, 2015; Kaye, Bradford, & Curtin, 2016).

Following this, participants reported their subjective response to a series of increasing intensity 200-ms-duration electric shocks administered to their fingers (Hogle et al., 2010). Shock intensity during the main task was set to each participant's subjective maximum tolerance threshold to minimize individual differences in sensitivity. Participants next completed the main task (see the Unpredictable–Predictable Stressor Task section). Participants then completed a self-report battery of individual difference measures. Once finished, participants were debriefed, paid, and released. Alcoholics were released only after their alcohol craving had returned to baseline.

Unpredictable–Predictable Stressor Task

Participants completed eight blocks of trials in the unpredictable–predictable stressor task (Hefner et al., 2013). In each block, participants viewed a series of cues (i.e., colored squares) presented in one of four block types: *predictable shock* blocks, *predictable no-shock* blocks, *unpredictable shock* blocks, and *unpredictable no-shock* blocks. Participants were instructed about the specific cue–shock contingencies in each block prior to task start. Participants completed two blocks of each block type in one of eight between-subjects counterbalanced task block orders. A message indicating block type was presented on the monitor at the onset of each block. The color of the cues varied across the four block types to further highlight the block type. The entire procedure required approximately 30 min to complete.

In the *predictable shock* blocks, participants were instructed that the duration of all cues was 5 s, separated by an intertrial interval (ITI; range = 10–20 s). They were instructed that each cue would coterminate with an electric shock (.25 s prior to cue offset) and that no shocks would be administered at any other time. Therefore, shock administration was temporally predictable and imminent following cue onset (4.75 s after each cue) in these blocks. A total of 10 predictable shock cues were presented.

In *unpredictable shock* blocks, participants were instructed that the duration of cues would vary from 5 s to 3 min, separated by an ITI (range = 10–20 s). In fact, four discrete cue durations were used (5, 20, 50, and 80 s). They were instructed that each cue would coterminate with an electric shock (.25 s prior to cue offset). Therefore, given that the duration of these cues was unknown, shock administration was temporally unpredictable following cue onset in these blocks. A total of 12 unpredictable cues (three times per duration) were presented.[1]

We also included two *predictable* and two *unpredictable no-shock* blocks. All parameters (e.g., number of cues, cue duration) were identical to those of their matched shock blocks. However, participants were instructed that no shocks would be administered at any time during these no-shock blocks. These blocks were included as a nonaversive control condition from which to calculate startle potentiation in shock blocks.

Measures

Startle response. We measured electromyographic (EMG) startle response to acoustic probes (50 ms of 102 dB white noise with near instantaneous rise time) administered 4.5 s after cue onset during both predictable and unpredictable cues and at later times 19.5, 49.5, and 79.5 s during unpredictable cues. A total of 24 probes (six times each) were presented at 4.5 s post cue onset during a subset of predictable and unpredictable shock and no-

[1] The differing number of cues across predictable and unpredictable conditions followed from design decisions. Specifically, we wanted to match the number of startle probes in the two primary conditions (six probes during predictable shock cues and six probes at 4.5 s into unpredictable shock cues). However, we also included an additional six probes at later time points in the unpredictable shock cues (two probes each at 19.5, 49.5, and 79.5 s) to allow us to test whether group differences in startle potentiation persisted during unpredictable shock cues. As such, two more unpredictable cues (12 total) were needed to allow for these additional startle probes during unpredictable cues to assess responding at later time points.

shock cues in the main task. Twelve probes (two times each) were presented at 19.5, 49.5, and 79.5 s post cue onset during a subset of the longer unpredictable shock and no-shock cues. An additional 24 probes were presented during ITIs across all blocks to decrease probe predictability. Three probes were also presented at the start of this procedure to habituate the nonlinear portion of the startle response. Habituation and ITI probes were not included in any analyses. Serial position of the probes was counterbalanced within-subject.

We recorded EMG response to the acoustic startle probes from two 4-mm Ag-AgCl sensors placed according to published guidelines beneath the right eye over the orbicularis oculi muscle (Blumenthal et al., 2005; van Boxtel, Boelhouwer, & Bos, 1998). We sampled EMG activity at 2500 Hz with an online bandpass filter (1–500 Hz) using NeuroScan SynAmps bioamplifiers and Scan (Version 4.3). We performed offline processing in Matlab using EEGLab (Delorme & Makeig, 2004) and PhysBox plugins (Curtin, 2011). This processing included epoching (−50 to 250 ms surrounding probe), high pass filtering (28 Hz, 4th-order Butterworth, zero phase shift), smoothing (signal rectification followed by 30 Hz, 2nd-order Butterworth low-pass filter, zero phase shift), and baseline correction. Startle magnitude was scored as the peak response between 20 and 100 ms post probe onset. We rejected trials containing artifact consistent with standard practices from our laboratory (Kaye et al., 2016). This included trials with deflections greater than ±20 μV in the 50-ms preprobe baseline (i.e., unstable baseline) and trials with mean activity ≤−10 μV between 150 and 250 ms post probe onset (i.e., baseline overcorrection due to preepoch artifact). Startle potentiation was calculated as the increase in startle magnitude during shock cues relative to no-shock cues in matched blocks. We tested our primary prediction using startle potentiation at 4.5 s to allow for a matched comparison across unpredictable and predictable cues. We evaluated startle potentiation at the later probe times during unpredictable cues to examine the stability of the primary effect.[2]

Self-report measures. All participants completed a self-report battery including demographic information, Beck Anxiety Inventory (BAI; Beck & Steer, 1990), Beck Depression Inventory (BDI; Beck & Steer, 1987), Trait Fear-55 scale (Vizueta, Patrick, Jiang, Thomas, & He, 2012), and Rutgers Alcohol Problems Index (White & Labouvie, 1989). We used the SCID–RV to determine alcohol-dependence diagnoses, presence of a withdrawal syndrome (absent, subthreshold, or present), and duration of abstinence. We assessed quantity of alcohol use (drinks per 28 days) with the Timeline Follow-Back.[3]

Results

We accomplished data analysis and figure preparation with R (R Development Core Team, 2015) within RStudio (RStudio, 2016) using the *lmSupport* (Curtin, 2015) package.

Sample Characteristics by Group

We report and test group differences for sample characteristics in Table 1. The groups were comparable on age, sex, race, ethnicity, and startle reactivity. As expected, significant group differences were observed for quantity of alcohol use and alcohol-related problems. In addition, the two groups were significantly different on trait fear, anxiety, and depression.

Startle Potentiation During Unpredictable Versus Predictable Stressors

We analyzed startle potentiation at 4.5 s post cue onset in a general linear model (GLM) with a between-subjects regressor for group (alcoholic vs. control) and repeated measures for stressor type (unpredictable vs. predictable). These GLMs included task block order and startle reactivity following published recommendations and our standard laboratory practices (Bradford et al., 2014). We also included measures of anxiety and depression (i.e., BAI, BDI) as covariates to increase power, given their empirically

[2] We calculated Spearman-Brown–corrected split half (odd vs. even trials) internal consistency for startle magnitude in all conditions at 4.5 s post cue onset (unpredictable no-shock r_{sb} = .96, predictable no-shock r_{sb} = .93, unpredictable shock r_{sb} = .95, predictable shock r_{sb} = .96) and later times in unpredictable cues (unpredictable no-shock r_{sb} = .95, unpredictable shock r_{sb} = .94). We calculated Spearman-Brown–corrected split half internal consistency for startle potentiation at 4.5 s post cue (unpredictable r_{sb} = .64, predictable r_{sb} = .37) and at later times during unpredictable cues (r_{sb} = .52).

[3] All participants also completed the Short Michigan Alcohol Screening Test (Selzer, Vinokur, & van Rooijen, 1975), Fagerström Test for Nicotine Dependence (Heatherton, Kozlowski, Frecker, & Fagerström, 1991), Externalizing Spectrum Inventory–100 (Venables & Patrick, 2012), Childhood Trauma Questionnaire—Short Form (Bernstein et al., 2003) and a report of the typical quantity and frequency of their alcohol use. Alcoholic participants also completed the Alcohol Dependence Scale (Skinner & Horn, 1984) and a version of the clinician-administered Clinical Institute Withdrawal Assessment for Alcohol—Revised (Sullivan, Sykora, Schneiderman, Naranjo, & Sellers, 1989) that was modified for self-report. These measures were collected as part of a standard battery of questionnaires used in our laboratory for aims not directly relevant to the stress neuroadaptation thesis tested in this study. Finally, we recorded several peripheral physiology measures for future exploratory analyses not relevant to the current study. These measures are corrugator supercilii EMG, heart rate, and skin conductance.

Participants also completed the Short form of the Positive and Negative Affect Schedule (Short PANAS; Mackinnon et al., 1999) during the experimental session to measure current mood, independent of the primary unpredictable stressor task. Participants completed the PANAS–X at four times during the experimental session: (a) prior to the initial startle reactivity assessment, (b) after the shock sensitivity assessment, (c) midpoint of the unpredictable stressor task, and (d) after the unpredictable stressor task. We analyzed the PANAS–X Negative Affect subscale in a GLM with a between-subjects regressor for group (alcoholic vs. control) and repeated measures for time (baseline vs. postshock sensitivity vs. midtask vs. posttask). There was a significant effect of time, $F(3, 318) = 4.33$, $p = .005$, consistent with expected general increases in self-reported state negative affect after receiving electric shock. However, there was no significant main effect of group, $t(106) = .77$, $p = .441$, or Group × Time interaction, $F(3, 318) = .79$, $p = .499$. We did not expect to observe group differences on this measure for two reasons. First, the PANAS–X assesses current mood rather than phasic reactivity to the stressors included in the stressor task. Rodent affective neuroscience indicates that stress neuroadaptations in the CRF and NE mechanisms that are putatively indexed by startle potentiation to unpredictable stressors support "dynamic, active response to an acute stressor" rather than tonic, persistent negative mood states (Koob & Zorrilla, 2012, p. 309; also see Heilig, Goldman, Berrettini, & O'Brien, 2011). Second, the PANAS–X was not used to assess reactivity selectively to unpredictable stressors but rather overall mood state at various points during the experimental session.

Table 1
Descriptive Statistics for Demographic, Affective, and Drinking Related Individual Difference Measures by Group

Variable	Control		Alcoholic		
	M (*SD*)	%	*M* (*SD*)	%	*p*
Demographics					
Age (in years)	43.5 (9.0)		43.7 (11.6)		.883
Sex (male)		71.9		70.7	1.000
Race					.386
African American		5.3		15.5	
Native American		1.8		1.7	
Asian		1.8		.0	
Caucasian		89.5		81.0	
Other		1.8		1.7	
Hispanic		3.5		5.2	1.000
Affective individual differences					
Trait fear	48.7 (20.7)		61.1 (25.5)		.005**
Beck Anxiety Inventory	3.6 (5.7)		9.2 (8.2)		<.001***
Beck Depression Inventory	4.6 (7.5)		10.1 (6.9)		<.001***
Startle reactivity	111.1 (84.1)		128.4 (92.8)		.297
Alcohol use and problems					
Quantity of alcohol use (drinks/28 days)[a]	11.9 (14.5)		204.3 (134.5)		<.001***
Alcohol-related problems[b]	2.2 (4.3)		53.5 (18.2)		<.001***
Duration of abstinence (days)			32.4 (14.4)		
Withdrawal syndrome					
Absent				15.5	
Subthreshold				19.0	
Present				65.5	

Note. Internal consistency of all self-report questionnaires of affective individual differences and alcohol-related problems was excellent, including trait fear (Cronbach's $\alpha = .94$), Beck Anxiety Inventory ($\alpha = .94$), Beck Depression Inventory ($\alpha = .92$), and Rutgers Alcohol Problem Index ($\alpha = .98$). $N = 115$ (58 alcoholics).
[a] Determined by Timeline Follow-Back (Sobell & Sobell, 1992) for the most recent 28 days for controls and the last 28 days preceding cessation for alcoholics. [b] Assessed with the Rutgers Alcohol Problems Index (White & Labouvie, 1989).
** $p < .01$. *** $p < .001$.

verified relationship with startle potentiation in our task.[4] We excluded one GLM model outlier (i.e., studentized residual with Bonferroni corrected $p < .05$; J. Fox, 1991) from all analyses involving startle potentiation as the dependent measure. We include partial eta-square (η_p^2) and raw GLM parameter estimates (b) to document effect size. We present descriptives for startle magnitude and startle potentiation for all conditions in Table 2.

We first confirmed, as manipulation checks, significant overall startle potentiation ($\eta_p^2 = .61$, $b = 29.9$, 95% confidence interval [CI: 24.5, 35.2]), $t(80) = 11.11$, $p < .001$, indicating that the shock stressors elicited robust defensive reactivity. Startle potentiation was also significant separately for unpredictable stressors ($\eta_p^2 = .55$, $b = 31.5$, 95% CI [25.1, 37.9]), $t(80) = 9.80$, $p < .001$, and predictable stressors ($\eta_p^2 = .50$, $b = 28.2$, 95% CI [21.9, 34.5]), $t(80) = 8.89$, $p < .001$. There was not a significant overall effect of stressor type on startle potentiation ($\eta_p^2 = .01$, $b = 3.3$, 95% CI [−3.6, 10.2]), $t(80) = .96$, $p = .341$.

As predicted, the interaction between group and stressor type was significant for startle potentiation ($\eta_p^2 = .06$, $b = 17.3$, 95% CI [2.5, 32.1]), $t(80) = 2.32$, $p = .023$ (see Figure 1). This interaction indicates that the size of startle potentiation during unpredictable (vs. predictable) stressors was greater in the alcoholics relative to controls. Within-subject tests of stressor type simple effects indicated that alcoholics displayed significantly greater startle potentiation during unpredictable than predictable stressors ($\eta_p^2 = .06$, $b = 11.9$, 95% CI [1.8, 22.1]), $t(80) = 2.35$, $p = .021$. In contrast, controls displayed comparable startle potentiation across both stressor types ($\eta_p^2 = .01$, $b = -5.3$, 95% CI [−15.4, 4.8]), $t(80) = 1.05$, $p = .297$.[5]

[4] Covariates are an important tool to increase power to test focal group effects in clinical and other research. We evaluated all individual difference measures from the demographics and affect sections of Table 1 as potential covariates. We did not consider individual difference variables related to alcohol use–problems as covariates, because these variables are fundamentally related to the focal group variable and therefore their variance should not be removed from primary analyses (Miller & Chapman, 2001). Covariates were selected if we confirmed that the specific covariate significantly predicted either overall startle potentiation or the selective increase in startle potentiation to unpredictable (vs. predictable) cues in GLMs that included only the task order and startle reactivity factors. critically, group was not included in these covariate selection analyses to avoid biasing selection of covariates by their relationship with group.

[5] Tests of between-groups simple effects are often not appropriate to decompose an interaction, particularly when preexisting differences in nonmanipulated grouping variables may confound these simple effects (Rosnow & Rosenthal, 1995). Nonetheless, we still report these simple group effects here for the interested reader. Specifically, the simple effect tests of group on startle potentiation were not significant during either unpredictable stressors ($\eta_p^2 < .01$, $b = 6.0$, 95% CI [−7.8, 19.8]), $t(80) = .86$, $p = .390$, or predictable stressors ($\eta_p^2 = .03$, $b = -11.3$, 95% CI [24.9, 2.3]), $t(80) = 1.65$, $p = .103$.

Table 2
Descriptive Statistics for Startle Magnitude and Potentiation by Stressor Type, Startle Probe Time, and Group

	Predictable: Early			Unpredictable: Early			Unpredictable: Late		
Group	No-shock	Shock	Potentiation	No-shock	Shock	Potentiation	No-shock	Shock	Potentiation
Alcoholic	87.9 (4.1)	110.4 (5.8)	22.6 (4.7)	82.5 (3.5)	117.0 (5.6)	34.5 (4.7)	80.3 (3.9)	121.9 (5.2)	41.5 (5.1)
Control	82.5 (4.1)	116.4 (5.8)	33.9 (4.7)	84.8 (3.4)	113.3 (5.6)	28.5 (4.7)	78.9 (3.9)	121.4 (5.2)	42.5 (5.1)

Note. Data represent point estimates (and standard errors) for startle magnitude during no-shock and shock cues and startle potentiation (i.e., the difference in startle magnitude between shock and no-shock cues) for early predictable, early unpredictable, and late unpredictable conditions from the general linear model (GLM). This GLM adjusted for all covariates, including task order, startle reactivity, Beck Anxiety Inventory, and Beck Depression Inventory (quantitative variables are mean-centered).

We conducted and report robustness analyses to evaluate the predicted Group × Stressor Type interaction in several plausible alternative analyses that could have been conducted to test this effect. This allows for increased confidence that conclusions about this interaction are not dependent on selection of any one specific analytic strategy. First, we believe we reported the strongest test of our primary hypothesis by using only the 4.5-s startle probe time for unpredictable cues because this probe time matches the only probe time used for the predictable cues. Nonetheless, the Group × Stressor Type interaction remained significant and of comparable size in an alternative analysis that contrasted mean startle potentiation across the four probe times in unpredictable cues (4.5, 19.5, 49.5, and 79.5 s) versus predictable cues ($\eta_p^2 = .05$, $b = 13.8$, 95% CI [.3, 27.3]), $t(80) = 2.03$, $p = .045$.

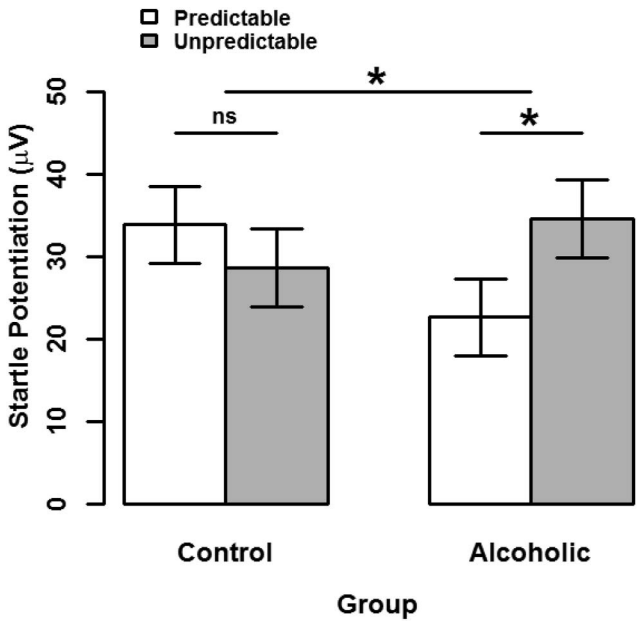

Figure 1. Startle potentiation by group and stressor type. Bars display startle potentiation to predictable (white) and unpredictable (gray) shock within each group (alcoholic vs. control). Confidence bars represent ±1 standard error for point estimates of startle potentiation from the general linear model (GLM). This GLM adjusted for all covariates including task block order, startle reactivity, Beck Anxiety Inventory, and Beck Depression Inventory (quantitative variables are mean-centered). The unpredictable versus predictable startle potentiation contrast was greater among alcoholics than controls ($p = .022$). Moreover, this simple effect contrast was significant among alcoholics ($p = .021$) but not controls ($p = .291$). * $p < .05$. Figure © 2016 by John Curtin, Daniel Bradford, Jesse Kaye, and Christine Moberg under Creative Commons Attribution 4.0 International Public License CC-By.

Second, covariates provide an important tool to increase statistical power and the precision of parameter estimation (Miller & Chapman, 2001). We identified and used two covariates in the analyses of startle potentiation in the primary analyses (Beck Anxiety Inventory, Beck Depression Inventory; see footnote 3). However, the Group × Stressor Type interaction remained significant and of comparable size without the Beck Anxiety Inventory ($\eta_p^2 = .05$, $b = 15.8$, 95% CI [.9, 30.7]), $t(81) = 2.10$, $p = .039$, or the Beck Depression Inventory ($\eta_p^2 = .06$, $b = 17.4$, 95% CI [2.8, 32.0]), $t(81) = 2.37$, $p = .020$, in the model. Furthermore, we confirmed that neither the Beck Anxiety Inventory nor the Beck Depression Inventory moderated the Group × Stressor Type interaction (*p*s = .481 and .886, respectively). In our primary analysis, we selected only covariates that were significant predictors of startle potentiation. Age, race, and trait Fear were not selected, because each had only marginal ($.10 < p < .05$) effects. However, the Group × Stressor Type interaction remained significant and of comparable size if we included all three of these additional measures in the model ($\eta_p^2 = .05$, $b = 15.1$, 95% CI [.0, 30.1]), $t(74) = 1.99$, $p = .050$.

Third, we identified and removed one GLM model outlier from the primary analyses of startle potentiation. Standard practice in our laboratory is to trim (i.e., remove) model outliers from all analyses because they excessively influence the standard errors of parameter estimates and therefore negatively impact statistical power and parameter estimation precision (Bradford et al., 2013; Hefner et al., 2013; Kaye et al., 2016). However, the Group × Stressor Type interaction remained significant and of comparable size when we retained this outlier in the analyses but winsorized it to reduce its influence (Keselman, Algina, Lix, Wilcox, & Deering, 2008; Wilcox & Keselman, 2003; $\eta_p^2 = .05$, $b = 16.2$, 95% CI [.8, 31.6]), $t(81) = 2.09$, $p = .040$.

Fourth, task order did not moderate the Group × Stressor Type interaction in the primary analysis, $F(7, 80) = 1.07$, $p = .391$. This indicates that the magnitude of the Group × Stressor Type interaction does not vary across task orders. In addition, the Group × Stressor Type interaction remained significant ($\eta_p^2 = .04$, $b = 14.2$, 95% CI [.2, 28.3]), $t(94) = 2.01$, $p = .048$, in an alternative analysis where we modeled task order as additive with respect to all effects of group.

Finally, although not technically a robustness analysis, separate analyses of startle magnitude during the no-shock cues were also conducted to confirm that the Group × Stressor Type interaction for startle potentiation did not result from group differences during the no-shock cues. There were no main effects of group on startle magnitude during no-shock cues overall ($\eta_p^2 < .01$, $b = 3.6$, 95% CI [−19.1, 26.2]), $t(80) = .31$, $p = .756$, or separately during unpredictable no-shock cues ($\eta_p^2 < .01$, $b = -2.2$, 95% CI [−12.3,7.8]), $t(80) = .44$, $p = .658$, or predictable no-shock cues ($\eta_p^2 < .01$, $b = 5.3$, 95% CI [−6.6, 17.3]), $t(80) = .89$, $p = .378$. Equally important, the Group × Stressor Type interaction was not significant for startle magnitude during the no-shock cues ($\eta_p^2 < .01$, $b = -3.7$, 95% CI [−12.3, 5.0]), $t(80) = .85$, $p = .400$.

Startle Potentiation Across Time During Unpredictable Stressors

We expected that the group effect for startle potentiation at 4.5 s during the unpredictable stressors would remain stable at later time points probed within the unpredictable stressors. To test this, we analyzed startle potentiation during unpredictable stressors in a GLM with a between-subjects regressor for group (alcoholic vs. control) and repeated measures for time (4.5 s vs. mean of 19.5, 49.5, 79.5 s). All covariates were included as described earlier. Startle potentiation during unpredictable stressors was significant at the later probe times ($\eta_p^2 = .64$, $b = 42.0$, 95% CI [35.0, 48.9]), $t(80) = 12.01$, $p < .001$, and significantly greater at the later probe times than at 4.5 s ($\eta_p^2 = .11$, $b = 10.4$, 95% CI [3.9, 17.0]), $t(80) = 3.17$, $p = .002$. However, the Group × Time interaction was not significant ($\eta_p^2 = .01$, $b = -6.9$, 95% CI [−21.1, 7.2]), $t(80) = .98$, $p = .332$, suggesting that group effects for unpredictable stressors were comparable across the early versus later probe times.

Potential Causes, Correlates, and Consequences of Increased Unpredictable Startle Potentiation

In separate GLMs using only alcoholics, we analyzed startle potentiation with between-subjects regressors for each target individual difference variable and repeated measures for stressor type (unpredictable vs. predictable). These models included all covariates as described earlier. We focused on Individual Difference × Stressor Type interactions because they indicated that the size of the selective increase in startle potentiation during unpredictable (vs. predictable) stressors varied significantly by that individual difference.

We observed a significant effect for alcohol-related problems such that the unpredictable (vs. predictable) startle potentiation contrast was greater among alcoholics who reported more alcohol-related problems ($\eta_p^2 = .10$, $b = .6$, 95% CI [.0, 1.2]), $t(39) = 2.11$, $p = .041$ (see Figure 2, Panel A). We also observed a significant linear effect for the withdrawal syndrome such that the selective increase in startle potentiation during unpredictable (vs. predictable) stressors increased as alcoholics reported a more substantial withdrawal syndrome (i.e., present > subthreshold > absent; $\eta_p^2 = .13$, $b = 33.2$, 95% CI [4.6, 61.8]), $t(38) = 2.35$, $p = .024$ (see Figure 2, Panel B). No significant interactions were observed for duration of abstinence or quantity of alcohol use ($ps = .587$ and .208, respectively; see Figure 2, Panels C and D).[6]

Discussion

In this study, we observed the predicted sensitized response to unpredictable stressors in human abstinent alcoholics, which manifested as selectively elevated startle potentiation to unpredictable (vs. predictable) stressors. Equally important, the contrast between unpredictable and predictable stressor startle potentiation used here implicates a stress neuroadaptation in the same CRF and NE mechanisms in the extended amygdala proposed by rodent behavioral neuroscience research. Future research in humans can strengthen this latter claim about mechanism by direct pharmacologic manipulation of these neurotransmitter systems while measuring unpredictable stressor startle potentiation in AOD-dependent users. However, research using such pharmacological manipulations must also address inherent limitations associated with systemic drug administration in humans (e.g., dose selection, blood–brain barrier penetration).

Research that pharmacologically manipulates relevant neurotransmitter systems in humans can also simultaneously document the treatment efficacy for these pharmacotherapies to ameliorate stress-induced relapse regardless of its etiologic origin. In fact, NE alpha1 antagonists and novel CRF antagonists have all generated substantial interest recently for their treatment potential (Koob & Zorrilla, 2012; Simpson et al., 2015; but see Kaye et al., in press, for a recent review). Of course, more precise targeting of sources and coping strategies for unpredictable stressors may one day increase the efficacy of psychological interventions as well.

Potential Mechanisms of Stress Neuroadaptation

Koob and others have proposed that a sensitized stress response results, in part, from a between-systems stress neuroadaptation where CNS stress system circuits are repeatedly recruited and strengthened to offset drug effects within the reward system following opponent-process principles (Koob & Le Moal, 2008b; Solomon & Corbit, 1973). In rodents, this mechanism is proposed to operate broadly for many addictive drugs beyond alcohol. We have now observed, consistent with the cross-drug thesis from rodent models, preliminary evidence for a sensitized response to unpredictable stressors among abstinent alcoholics (in the current study), 24-hr nicotine-deprived smokers (Hogle et al., 2010), and heavy daily marijuana users (Hefner, Starr, & Curtin, 2016).

Between-systems neuroadaptations provide one set of etiologic mechanisms for sensitized response to unpredictable stressors in addiction. Within-system neuroadaptations, where the primary cellular response within a specific system adapts to neutralize the drug's effects (Koob & Le Moal, 2008b) within that same system, may also contribute to a sensitized response to unpredictable

[6] We focused our analyses of individual difference moderators on four specific individual differences that were relevant to the stress neuroadaptation model. Three (alcohol related problems, withdrawal, and quantity of alcohol use) of these four individual differences were expected to significantly moderate the unpredictable versus predictable startle potentiation contrast. The size of this contrast was not expected to vary by duration of abstinence. Given our focus on only a few candidate moderators, we did not correct the p values from these analyses for multiple comparisons. A false discovery rate correction for the three predicted significant moderators yielded p values of .082, .082, and .587, respectively (Benjamini & Hochberg, 1995). As such, the effects of these moderators should be interpreted cautiously pending replication in independent samples.

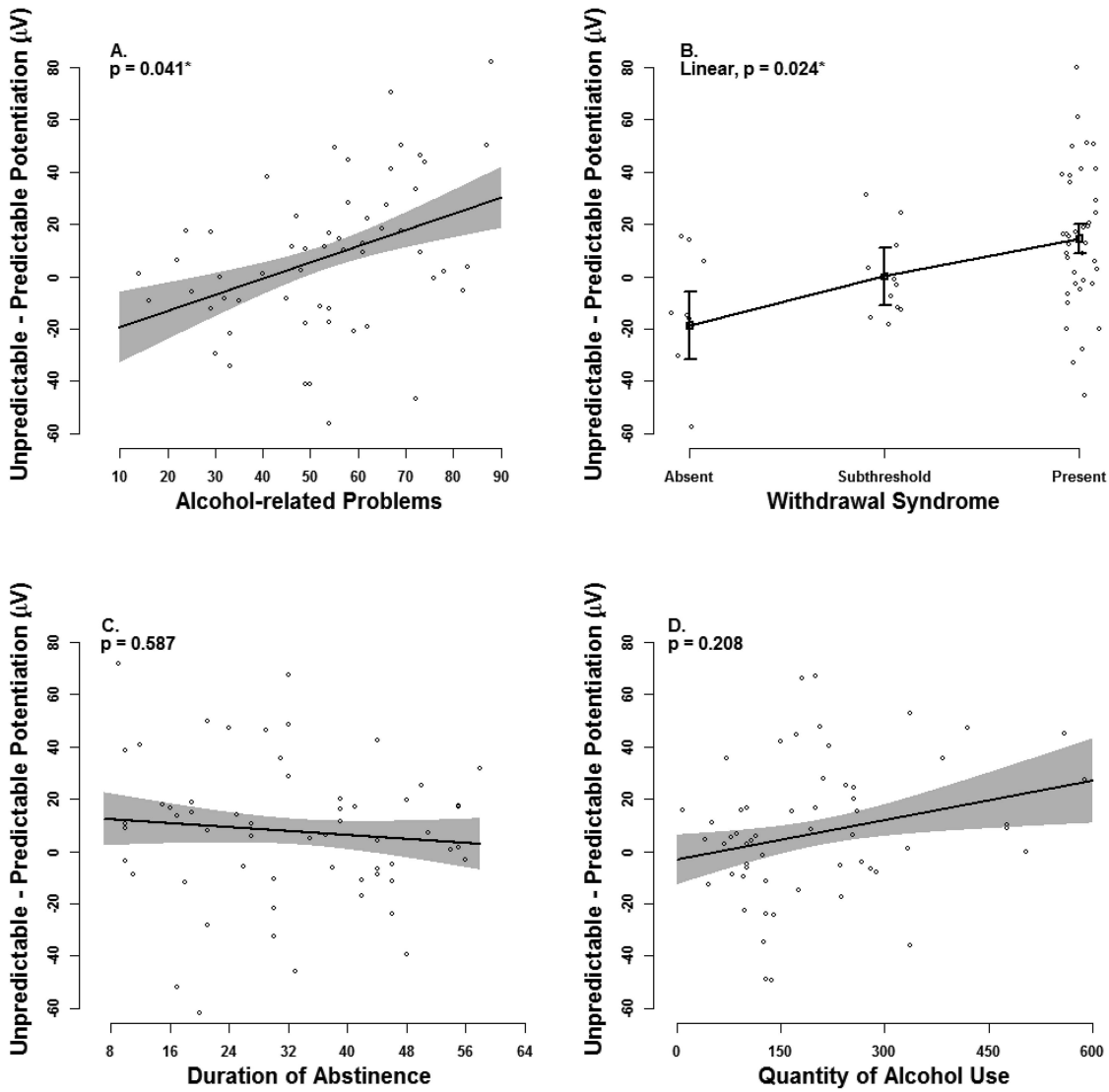

Figure 2. Individual Differences for unpredictable (vs. predictable) startle potentiation among alcoholics. Black lines display the relationship between the size of the unpredictable minus predictable startle potentiation difference score and each individual difference variable within the alcoholic group. Gray confidence bands (in Panels A, C, D) and black confidence bars (in Panel B) represent ±1 standard error for point estimates of startle potentiation from the general linear model (GLM). This GLM adjusted for all covariates including task block order, startle reactivity, Beck Anxiety Inventory, and Beck Depression Inventory (quantitative variables are mean-centered). * $p < .05$.

stressors. Whereas Koob et al. have discussed neuroadaptations within the reward system in depth (e.g., Koob & Le Moal, 2008b), the current study combines with other data from our laboratory to implicate a possible within-system adaptation in the stress system to repeated alcohol exposure (Kaye et al., in press). Specifically, across a programmatic series of experiments (Bradford et al., 2013; Hefner & Curtin, 2012; Hefner et al., 2013; Moberg & Curtin, 2009), we have demonstrated that acute administration of alcohol selectively reduces startle potentiation to unpredictable (vs. predictable) stressors in humans (see Bradford et al., 2013, for a discussion of implications for stress response–dampening theory). Thus, allostatic neuroadaptations to repeated alcohol stress response dampening may also contribute to the compensatory sensitized response to unpredictable stressors observed in abstinent alcoholics in this study. We hope these preliminary observations encourage reverse translational studies to search for the neural mechanisms of this potential within-stress system adaption in rodent models (Koob, Lloyd, & Mason, 2009; Sinha, Shaham, & Heilig, 2011). Cross-sectional and longitudinal studies with rodents may measure startle potentiation after both acute and chronic alcohol administration to probe these opposing compensatory effects on stress response dampening and sensitization, respectively.

This behavioral neuroscience research may be most sensitive to detect stress neuroadaptations by focusing on the distinction between predictable versus unpredictable stressors. We and others have recently made calls for an increased focus on this critical feature of stressor predictability in refining rodent models of stress neuroadaptation and stress-induced reinstatement (Kaye et al., in press; Mantsch et al., 2016).

This study was initiated to test the rodent model thesis that chronic alcohol use would cause sensitized response to unpredictable stressors via stress neuroadaptation in human alcoholics. Our results are consistent with this thesis. However, the cross-sectional measurement of startle potentiation in preexisting groups of alcoholics and healthy controls allows for other plausible interpretations. For example, increased startle potentiation to unpredictable stressors may represent a premorbid risk factor for AOD use disorders rather than a consequence of chronic AOD use (Gorka, Lieberman, Phan, & Shankman, 2016; Rasmussen & Kincaid, 2015). In other research, Gorka, Nelson, and Shankman (2013) observed that participants with comorbid panic and alcohol use disorders displayed increased startle potentiation to unpredictable stressors relative to both participants with only panic disorder and healthy controls. They suggested that elevated startle potentiation in panic disorder may motivate heavy alcohol use that contributes to development of comorbid alcohol use disorder. Indeed, some participants in the current study had comorbid mental health disorders (e.g., depression, anxiety disorders), which increases the generalizability of our findings, but our study was not designed to examine comorbidity specifically. Clearly additional research including longitudinal designs is required to adjudicate between these and other competing interpretations.

We focused on the unpredictable versus predictable startle potentiation contrast to explicitly test for group differences selectively during unpredictable stressors over and above any possible differences in generic threat responding. However, the observed pattern of group means may represent independent contributions from both this selective increase in response to unpredictable (vs. predictable) stressors in alcoholics and other premorbid differences or neuroadaptations associated with reduced responding to predictable stressors. Whereas responses to unpredictable stressors are mediated by NE- and CRF-sensitive pathways through the lateral divisions of the central amygdala and BNST, responses to predictable stressors are mediated by an overlapping but separate pathway through the medial division of the central amygdala (mCeA). Of note, the BNST has inhibitory effects on the mCeA (Campeau et al., 1997; Grillon et al., 2015; Haufler, Nagy, & Pare, 2013), which can manifest as attenuated startle potentiation or other fear expression to predictable stressors (Grillon et al., 2015; Kim et al., 2013; Meloni, Jackson, Gerety, Cohen, & Carlezon, 2006; Walker, Miles, & Davis, 2009). If stress neuroadaptations lead to generally increased BNST activity in alcoholics, these individuals could display a somewhat attenuated response to predictable stressors due to increased inhibitory effects of the BNST on the mCeA. Grillon et al. (2015) recently demonstrated that, consistent with this possibility, administration of a CRF antagonist to healthy participants increased their startle potentiation to predictable stressors, presumably through decreased activation of the CRF-sensitive BNST.

Individual Differences in Possible Stress Neuroadaptation

Our secondary analyses of alcoholics' individual differences clarify the nature of this increased response to unpredictable stressors and highlights important next steps. To start, alcoholics who displayed greater unpredictable (vs. predictable) startle potentiation also reported more alcohol-related problems. Taken at face value, this relationship establishes unpredictable startle potentiation as clinically relevant. As such, it may serve as a marker of one dimension of addiction severity (Gorka et al., 2016). Furthermore, it may be that individuals who experience greater unpredictable startle potentiation may struggle more with stronger urges and difficulty controlling their use in key situations when problems begin to emerge. Future research should clarify the causal relationship between unpredictable startle potentiation and alcohol-related problems and measure possible mediators such as drinking urge.

Unpredictable (vs. predictable) startle potentiation was greater among alcoholics who reported a clinically significant withdrawal syndrome. This connects this effect with a cardinal diagnostic criterion for AOD use disorders (Baker et al., 2004; Heilig, Egli, Crabbe, & Becker, 2010), the withdrawal syndrome. Given that negative affect is the motivational core of the withdrawal syndrome (Baker et al., 2004), it may be that a stress neuroadaptation contributes to both sensitized response to unpredictable stressors and withdrawal-related negative affect. In our study, we found no evidence that the relative increase in unpredictable (vs. predictable) startle potentiation varied as a function of duration of abstinence among alcoholics who had abstained from between 1 week and 2 months. This is consistent with other research that has suggested that stressors continue to instigate AOD relapse well into protracted abstinence in humans (Brown et al., 1990; McKay, 1999) and rodents (Mantsch et al., 2016). In contrast to the more transient physical symptoms of withdrawal, withdrawal-related negative affect may also be long-lasting and contribute to later relapse among some AOD users (Baker et al., 2004). Our study's cross-sectional design did not allow us to examine the temporal ordering of the increased unpredictable (vs. predictable) startle potentiation, alcohol use patterns, alcohol-related problems, and the emergence of the withdrawal syndrome. Future longitudinal research in humans can clarify issues related to the relative onset, developmental course, and persistence of these key features of AOD use disorders. In particular, we believe that questions about whether an activated withdrawal syndrome from acute deprivation is sufficient or even necessary to observe increased reactivity to unpredictable stressors are important to consider immediately (for detailed review of these issues see Kaye et al., in press).

Quantity of alcohol use in the 28 days prior to cessation did not predict the size of unpredictable (vs. predictable) startle potentiation in this study. If this sensitized stress response results from chronic alcohol use, it may be that a more comprehensive assessment aggregated over a longer time span is necessary to detect the impact of drinking quantity. Alternatively, use characteristics other than quantity may be more critical to its development. For example, rodent models have suggested that particular patterns of episodic drinking (e.g.,

repeated binging and withdrawal) rather than overall quantity may be necessary to promote allostatic changes in stress-related neurocircuitry (Griffin, Lopez, & Becker, 2009; O'Dell, Roberts, Smith, & Koob, 2004).

Of course, increased confidence in these individual differences as well as the mechanism(s) that account for increased unpredictable (vs. predictable) startle potentiation overall in abstinent alcoholics requires replication with varied research designs. We hope that such research proceeds in parallel with both humans and animal models as facilitated by the use of startle potentiation in cross-species translational research. Such a program of research holds high promise for rapid bidirectional translation between basic research on mechanism and applications in the pharmacologic and psychosocial treatment of AOD use disorders (Kaye et al., in press).

Bidirectional translation can also occur between laboratory and more naturalistic research on stressors in the "real world," with a different set of opportunities and challenges. For example, the stressors in the current task were temporally unpredictable, but real-world stressors may incorporate unpredictability in alternative, often complex ways. For this reason, we have developed alternative laboratory tasks that manipulate how predictable the stressor may be with respect to probability (Hefner & Curtin, 2012), intensity (Bradford et al., 2013), or location (Bradford, Motschman, Starr, & Curtin, 2017). These features (e.g., probabilistic and temporal uncertainty) can be combined to increase stressor unpredictability (Moberg & Curtin, 2009). This previous research has suggested that acute alcohol administration selectively reduces response when these stressors are unpredictable, regardless of its source, but the impact of chronic AOD use in these tasks has yet to be considered in a clinical sample. Other researchers have recently noted that the effects of alcohol on response to unpredictable stressors in social drinkers also appear to extend beyond manipulations of electric shock to more real-world situations that include inherent unpredictability, such as in most social interactions (e.g., Sayette, 2017; see Fairbairn & Sayette, 2014, for a review).

Other important characteristics of real-world stressors besides predictability can also be manipulated in the laboratory. For example, stressor intensity (Bradford et al., 2013; Moberg, Weber, & Curtin, 2011) or controllability (Bradford, Shireman, Schneck, & Curtin, n.d.; Maier, 2015) may have influences on AOD-related behavior. Stressors may become less predictable if appraisal processes are degraded by distractors, and this too can be modeled in the laboratory (e.g., Curtin, Patrick, Lang, Cacioppo, & Birbaumer, 2001). Finally, naturalistic research can complement these laboratory approaches by taking advantage of rapidly developing mobile technologies that allow for real-time measurement of subjective emotional response, behavior, and physiology combined with important contextual information provided by GPS location services and indices of peer-to-peer interactions in the real world (Curtin, Zhu, Gustafson, & Alagoz, 2015; Harari et al., 2016). All of these approaches can and should be marshaled to better understand and treat the contributions of unpredictable stressors to the etiology and maintenance of AOD use disorders.

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Anticipation of Smoking Sufficiently Dampens Stress Reactivity in Nicotine-Deprived Smokers

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Most smokers attempting to quit will relapse, even when using evidence-based cessation treatment. This illustrates the need for better understanding of the relapse process to thereby improve cessation treatments. Although the impact of stress sensitivity on relapse is clear, little research has precisely examined stress reactivity in addicted individuals. Further, most research on relapse focuses on affect surrounding self-administration, and does not address potentially important preconsumption processes such as anticipation of use. We examined the effects of anticipation and actual smoking on stress reactivity in 34 deprived smokers withdrawn for 24 hr and 37 nondeprived smokers, with 37 nonsmoker controls. Using a cued shock stressor task, we measured stress reactivity via startle potentiation and self-reported anxiety. After completing the task once, smokers anticipated smoking a cigarette resting in front of them while they completed the task a second time. Smokers then smoked before completing the task a third and final time. Nonsmokers anticipated and drank water as a control. Anticipation of smoking significantly attenuated both startle potentiation and self-reported anxiety to shock cues for deprived smokers relative to nondeprived smokers. Smokers' stress reactivity was not reduced by smoking beyond the prior effect of anticipation. These results suggest that anticipation, rather than actual drug consumption, may drive the primary reinforcing effect of reduced stress reactivity in smoking. Future research is needed to understand this effect of anticipation on drug use and to determine whether anticipation would make an effective intervention target for addiction and other psychopathology that exhibits increased stress sensitivity.

Keywords: stress sensitivity, addiction, anticipation of drug use, smoking, startle potentiation

It has been 50 years since the 1964 U.S. Surgeon General's report on the negative health consequences of smoking. Still, tobacco-related illnesses continue to cause the death of more than 480,000 annually in the United States alone (National Center for Chronic Disease Prevention and Health Promotion Office on Smoking and Health, 2014). Although the majority of smokers want to quit smoking, and more than half try to quit (Centers for Disease Control and Prevention, 2011), relapse remains the most common outcome, even for smokers who used evidence-based cessation treatment (Fiore et al., 2008). In order to develop more effective cessation treatments, we need a better understanding of the cascade of cognitive and affective processes that both precede and ultimately result in relapse. Such understanding could provide unique insight into the relapse process, which could be used to improve smoking cessation treatment as well as treatments for other drug addictions.

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Many addiction models suggest that stress plays an important role in smoking and other drug use (Kassel, Stroud, & Paronis, 2003). For instance, human–animal translational research has indicated that chronic drug use leads to adaptations in affect-related brain structures, resulting in increased stress sensitivity, which may manifest as exaggerated negative affect in response to stressors when in withdrawal (Hefner, Moberg, Hachiya, & Curtin, 2013; Koob & Volkow, 2010). Consistent with translational research, negative reinforcement models of addiction posit that alleviation of negative affect is a primary motivator for drug use (Baker, Piper, McCarthy, Majeskie, & Fiore, 2004). Individual experiences also support the importance of stress sensitivity in maintaining addiction, as alleviation of the negative affective component of stress is commonly reported as a primary motive for smoking or other drug use (Novak, Burgess, Clark, Zvolensky, & Brown, 2003; Parrott, 1999). Furthermore, both animal and human data have led to models of "stress-induced relapse," which posit that the presentation of discrete, acute stressors motivates individuals in abstinence to again use drugs, perhaps because of the sensitivity these individuals have to stressors (Bossert, Marchant, Calu, & Shaham, 2013; Koob & Volkow, 2010; Sinha, 2001).

Despite clear demonstration of increased sensitivity to stressors, stress-induced relapse, and smokers' beliefs that smoking will reduce their negative affect, direct effects of smoking on stress responses have been difficult to demonstrate in the laboratory (Kassel et al., 2003). Our study was designed to explore two potential reasons for this inconsistency. First, there is often a lack of nuance in the experimental measurement of stress reactivity in

addiction, as many studies have relied on complicated stressors and/or measures of stress that do not take advantage of recent findings on neural mechanisms afforded by animal–human translational stress and addiction research (Curtin & Lang, 2007; Kassel et al., 2003; Perkins, Karelitz, Conklin, Sayette, & Giedgowd, 2010). Second, the experimental study of stress response in addiction is usually limited to the impact of stressors on actual consumption, and vice versa, despite the potential importance of stress reactivity in relation to other important drug use processes, such as anticipation of drug use.

Our research takes advantage of translational methods used to study the central nervous system (CNS) component of the stress response by using potentiation of the defensive startle reflex to cued threat of electric shock (Bradford, Shapiro, & Curtin, 2013; Davis, 2006; Hefner et al., 2013; Hogle, Kaye, & Curtin, 2010). The primary startle circuit is directly modulated through projections from some of the same brain structures implicated in neuroadaptation addiction models (e.g., the extended amygdala; Davis, 2006; Koob & Volkow, 2010). Startle potentiation is a direct measure of defensive reactivity to stressors (i.e., stress reactivity), which is congruent with negative affect and not simply arousal (Davis, 2006; Vaidyanathan, Patrick, & Cuthbert, 2009). The startle response is resistant to volitional control and is less susceptible to responder bias than self-report measures of stress reactivity in addiction (Kassel et al., 2003). Our research also uses threat of shock, which is a robust, discrete, and reliable stressor used to elicit negative affect across species, thus providing a translational bridge for stress-addiction research (Engelmann, Radke, & Gewirtz, 2009; Hogle et al., 2010; Koob & Volkow, 2010). A few other studies have used startle potentiation to measure negative affect in responses to stressors (i.e., threat of electric shock) in smokers (Grillon, Avenevoli, Daurignac, & Merikangas, 2007; Hogle & Curtin, 2006; Hogle et al., 2010), but these have focused on the impact of drug deprivation and consumption on stress reactivity.

Stress-addiction research has traditionally focused on connections between stress and consumption of the drug, thus bypassing any processes that lead up to that consumption. Relapse is a process with identified components (e.g., initial cessation, lapse, relapse; Shiffman et al., 2006); however, it is possible to further parse a single lapse event into subcomponents. For example, recent work using drug cue-availability paradigms suggests that the period before imminent drug use may involve a host of cognitive, affective, and attentional changes (Carter & Tiffany, 2001; Robinson et al., 2014). We refer to this phenomenon immediately prior to drug use as "anticipation." Anticipation of smoking could provide an ideal target for intervention, because the smoker has not yet lapsed (i.e., succumbed to that first cigarette postquit)—almost certainly the death knell in a cessation attempt (Kenford et al., 1994).

The goals of this study were to examine the effects of anticipation and subsequent smoking on stress reactivity in smokers. Specifically, we tested (a) the effect of anticipation of smoking on stress reactivity among deprived smokers relative to nondeprived smokers, and (b) the effect of cigarette consumption on stress reactivity beyond anticipation of smoking among deprived smokers relative to nondeprived smokers. To examine these effects, smokers completed a stressor task at baseline, in anticipation of smoking and then after smoking. To control for extraneous variables, such as anticipation of consumption, broadly defined, or other smoker characteristics, we ran an additional sample of nonsmokers who simply anticipated then drank water. We measured participants' stress reactivity by measuring the potentiation of their defensive startle reflex during threat of shock using a modified version of the no-shock, predictable-shock, unpredictable-shock (NPU) cued threat task (Schmitz & Grillon, 2012). The NPU task allowed us to assess startle potentiation to threat generally as well as unpredictable and predictable subtypes, as selective sensitivity to unpredictable threat is an important component of some models of drug addiction (Hefner et al., 2013; Hogle et al., 2010; Koob & Volkow, 2010). We also assessed self-reported anxiety to index participants' subjective stress reactivity to the threat cues (Bradford et al., 2013).

Method

Recruitment and Screening

We recruited 84 daily smokers and 45 nonsmokers from the greater Madison, Wisconsin, community via newspaper, web, and TV advertisements. Following a phone screen, eligible potential participants attended an in-person session to further assess eligibility, learn about the study, provide written informed consent, and complete self-report measures of demographics and smoking history. Smokers also completed the Fagerström Test of Nicotine Dependence (Heatherton, Kozlowski, Frecker, & Fagerström, 1991), a well-validated, six-item dependence measure.

Eligible participants were: ≥18 years of age, able to read and write in English, not currently using psychiatric medication, and reported no current physical or psychological condition that would contraindicate participation in the threat of shock task (e.g., recent heart problems, anxiety disorders). Eligible smokers also had to report smoking ≥10 cigarettes per day for at least 1 year, no current participation in any smoking cessation program or treatment, as well as provide a screening session exhaled carbon monoxide (CO) level ≥10 parts per million (ppm; Hogle et al., 2010; Piper & Curtin, 2006). Nonsmokers had to report smoking <100 cigarettes in their lifetime, no current or past daily cigarette use, and provide a CO level <10 ppm.

Smokers were stratified by gender and randomly assigned to either the deprived or the nondeprived smoker group. Deprived smokers abstained from all nicotine-containing products for 24 hr prior to their laboratory session, whereas nondeprived smokers maintained their typical cigarette usage pattern prior to their laboratory session.

Experimental Session

At the beginning of the experimental session, we measured CO level again to biochemically confirm self-reported abstinence (≤50% of screening CO level) among the deprived smokers. Deprived smokers who did not meet this criterion were rescheduled. If nondeprived smokers reported last smoking more than 30 min before the experimental session, we asked them to smoke a cigarette immediately prior to beginning the session to minimize withdrawal symptoms during the task. Smoking withdrawal symptoms were assessed prior to task start (Wisconsin Smoking Withdrawal Scale; Welsch et al., 1999).

Participants' general startle reactivity to acoustic startle probes was assessed while they viewed a series of nine colored squares (threat cues) presented on a computer screen for 5 s each with a variable intertrial interval (intertrial interval [ITI]; 14 s to 20 s; M = 17 s). Next we measured participants' subjective shock tolerance per standardized procedures from our laboratory (e.g., Curtin, Patrick, Lang, Cacioppo, & Birbaumer, 2001; Hogle et al., 2010; Moberg & Curtin, 2009). Participants reported their response to a series of increasing intensity, 200-ms duration electric shocks (7 mA maximum) administered across the distal phalanges of the index and ring fingers of participants' left hands. We stopped the procedure once participants reached the maximum level of shock they could tolerate. We set shock intensity during the main task to each participant's subjective maximum tolerance threshold to minimize individual differences in shock sensitivity.

Participants completed the cued threat task three times (see Cued Threat Task section for description). After the first run of the task, smokers were asked to take out one of their cigarettes and hold it while the experimenter set up some things in another room. Nonsmokers were given a bottle of water to hold. After 3 min, the experimenter reentered the room, told the participant they would be able to smoke (or drink) after the next run of the task, and placed the cigarette (or water) directly below the computer screen, where it remained during completion of the second run of the task. Next, participants were escorted outside to either smoke as much as they wanted (or drink water). We measured amount of cigarettes smoked to the nearest one quarter of the original tobacco rod of each cigarette (see Table 1). Approximately 15 min later, participants completed the task for the final time. Immediately after each run of the task, participants rated how anxious they were when they saw the threat and nonthreat cues on a 7-point rating scale (1 = *not at all anxious*; 7 = *extremely anxious*). We reimbursed all participants $20/hr for a total of approximately 4 hr in the laboratory, and deprived participants an additional $50 for adherence to the tobacco-deprivation criterion.

Cued Threat Task

Participants viewed a series of three colored square cues presented on a computer screen for 5 s each separated by a variable ITI (14s to 20 s; M = 17 s). These cues were presented in one of three block types: unpredictable shock blocks (U), predictable shock blocks (P), and no-shock blocks (N). A preblock message on the monitor informed participants of the next block type. In unpredictable shock blocks, participants were instructed that electric shocks could be administered at any point during the block, both during the cues and in the ITI. A total of six shocks were administered across two unpredictable shock blocks (two shocks at either 2 s or 4.8 s postcue onset, and four shocks during the ITI at 3 s, 6 s, or 9 s postcue offset). In the predictable shock blocks, participants were instructed that electric shocks would be administered only during the cues and that no shocks would ever be administered during the ITI. A total of six shocks were administered at 4.5 s postcue onset across two predictable shock blocks (i.e., during every cue; three shocks in each block). Three no-shock blocks were included as a nonaversive control condition from which to calculate startle potentiation during cues in predictable and unpredictable shock blocks. In no-shock blocks, participants were instructed that no shocks would be administered either during the cues or the ITIs. There were two block orders counterbalanced across participants: UNPNPNU and PNUNUNP. Startle potentiation was calculated as the increase in startle response to probes during cues in the shock blocks relative to cues in the no-shock blocks. Self-reported anxiety was analogously calculated as the increase in anxiety to the shock cues relative to the no-shock cues.

Table 1
Descriptive Statistics and Manipulation Checks for the Sample

	Deprived smokers	Nondeprived smokers	Nonsmokers
Demographics			
Total *N*	34	37	37
Female (%)	47	51	51
White (%)	50	70	73
High school or equivalent degree (%)[b]	44	57	84
Age	43.2 (11.2) range = 23–68	42.1 (11.8) range = 21–65	38.9 (15.5) range = 19–67
Screening session measures			
Cigarettes per day	17.1 (5.3) range = 10–30	18.1 (6.6) range = 10–40	—
Age of first cigarette	15.3 (3.7) range = 9–23	14.1 (3.0) range = 9–23	—
Years smoking daily	25.6 (10.6) range = 6–48	26.2 (11.1) range = 6–47	—
FTND	5.50 (1.6) range = 2–9	5.43 (2.2) range = 0–9	—
Screening session CO (ppm)[b]	19.0 (6.2) range = 10–39	20.0 (12.2) range = 10–62	2.1 (1.4) range = 0–7
Experimental session measures			
General startle reactivity (uV)	54.6 (38.0) range = 7–143	52.6 (44.8) range = 5–193	66.3 (43.5) range = 5–183
Experimental session CO (ppm)[a]	3.6 (1.7) range = 1–8	18.4 (7.1) range = 10–40	—
WSWS[a]	2.3 (0.5) range = 1–3	1.9 (.6) range = 1–3	—
Cigarettes during consumption	0.9 (0.4) range = 0.25–2	0.9 (0.3) range = 0.25–2	—

Note. Descriptive statistics (mean and standard deviation, unless otherwise noted) for manipulation checks for the entire final sample (N = 108). However, data recording errors led to missing "cigarettes smoked during consumption" data and "age of first cigarette" for three and one nondeprived smokers, respectively. Chi-square tests were used for qualitative variables (e.g., gender). T tests were used for quantitative variables (e.g., CO level). FTND = Fagerström Test of Nicotine Dependence; WSWS = Wisconsin Smoking Withdrawal Scale; ppm = parts per million.
[a] Dependent measures that displayed significant effects ($p < .05$) of deprivation (deprived vs. nondeprived smokers). [b] Dependent measures that displayed significant effects ($p < .05$) of smoker status (nondeprived smoker vs. nonsmoker).

Startle Response Measurement and Data Reduction

We used a bioamplifier (James Long Company, Caroga Lake, NY) to sample electromyographic activity in the orbicularis oculi muscle at 1,000 Hz from electrodes placed under the right eye, according to published guidelines (Blumenthal et al., 2005; van Boxtel, Boelhouwer, & Bos, 1998). We measured eyeblink startle response to 50-ms white-noise probes at 102 dB with near instantaneous rise time. During the general startle reactivity task, there were six noise probes during a subset of the visual cues at 3.5 s or 4.5 s postcue onset. There were 14 noise probes during a subset of the cues in the threat of shock (eight probes; four for each threat type) and no-shock (six probes) blocks at 3.5 s or 4.5 s, postcue onset, with equal probable timing. We presented additional noise probes during the ITIs in the general startle reactivity (three probes) and cued threat tasks (seven probes) to decrease probe predictability. A minimum of 13.5 s separated each probe from any previous startle eliciting event (i.e., another probe or electric shock). We matched serial position of the probes across block types within participants during the cued threat task.

Data reduction and processing followed published guidelines (Blumenthal et al., 2005). Specifically, offline processing included high-pass filtering (4th-order 28-Hz Butterworth filter), signal epoching (−50-ms to 250-ms period surrounding noise probe), rectification, and smoothing (4th-order 30-Hz Butterworth low-pass filter). Trials with greater than 40-μV deflections in the 50-ms preprobe baseline were rejected as artifacts (i.e., unstable baseline). We excluded 13 participants with <5 μV general startle reactivity (nonresponders) during data processing. These participants did not differ from startle responders on any of the variables listed in Table 1 ($ps > 0.05$). We scored peak eyeblink response between 20 ms and 100 ms postprobe onset relative to mean 50 ms preprobe baseline.

Analysis Plan

Data were analyzed using R (R Development Core Team, 2014). We analyzed startle potentiation and self-reported anxiety during cue presentations in separate general linear models (GLMs) each with a between-subjects factor for smoking group (deprived smokers, nondeprived smokers, nonsmokers), and repeated measures for task time (baseline, anticipation, consumption) and threat type (unpredictable, predictable). We followed up omnibus effects with planned contrasts using Fisher's least significant difference approach to protect against inflation of familywise error (Kirk, 1995). We analyzed the smoking-group factor using planned between-subject contrasts to examine the effects of deprivation (deprived smokers vs. nondeprived smokers) and smoker status (nondeprived smokers vs. nonsmokers). We analyzed the task-time factor using planned within-subject contrasts to examine the effects of anticipation (anticipation of smoking vs. baseline) and consumption (postconsumption vs. anticipation of smoking), consistent with our research goals. We included an interactive between-subjects regressor for general startle reactivity (mean centered) to control for individual differences in startle potentiation (Bradford, Kaye, & Curtin, 2014; Hogle et al., 2010; Schmitz & Grillon, 2012).[1] We report raw GLM coefficients (b) and partial eta squared (η_p^2) to document effect sizes.

Results

The final sample consisted of 34 deprived smokers, 37 nondeprived smokers, and 37 nonsmokers.[2] The three groups were comparable with respect to demographics and smoking variables, although nonsmokers were more educated than smokers (see Table 1). At the experimental session, deprived smokers reported significantly more withdrawal symptoms and provided significantly lower CO readings than nondeprived smokers (see Table 1).

The NPU task was successful in inducing stress, as indicated by significant (nonzero) startle potentiation, $b = 13.5$, $t(102) = 11.77$, $p < .001$, $\eta_p^2 = .57$, and significantly increased self-reported anxiety to threat cues, $b = 2.3$, $t(102) = 11.96$, $p < .001$, $\eta_p^2 = .58$, across smoking groups, task times, and threat types (see Table 2).

Analysis of startle potentiation revealed a significant smoking Group × Task Time interaction, $F(4, 204) = 3.35$, $p = .011$, $\eta_p^2 = .06$. Follow-up planned interaction contrasts revealed that anticipation of smoking had a greater dampening effect on startle potentiation for the deprived smokers than for the nondeprived smokers across threat types, $b = -10.4$, $t(102) = 2.43$, $p = .017$, $\eta_p^2 = .06$. Follow-up tests revealed that anticipation significantly attenuated startle potentiation for deprived smokers, $b = -10.9$, $t(102) = 3.55$, $p = .001$, $\eta_p^2 = .11$, but not nondeprived smokers, $b = -0.6$, $t(102) = 0.19$, $p = .851$, $\eta_p^2 < .01$, across threat types (see Figure 1).

Analysis of self-reported anxiety also revealed a significant smoking Group × Task Time interaction, $F(4, 204) = 2.44$, $p = .048$, $\eta_p^2 = .05$. Anticipation of smoking also had a greater dampening effect on self-reported anxiety for deprived smokers than for nondeprived smokers across threat types, $b = -0.6$, $t(102) = 2.01$, $p = .047$, $\eta_p^2 = .04$. Follow-up tests revealed that anticipation significantly attenuated self-reported anxiety for deprived smokers, $b = -0.8$, $t(102) = 3.34$, $p = .001$, $\eta_p^2 = .10$, but not nondeprived smokers across threat types, $b = -0.1$, $t(102) = 0.58$, $p = .563$, $\eta_p^2 < .01$ (see Figure 2).

As expected, no significant effects of smoker status (nondeprived smokers vs. nonsmokers) were observed for startle potentiation or self-reported anxiety ($ps > .05$). In addition, there were no significant effects of the consumption contrast (consumption vs. anticipation) or threat type (uncertain vs. certain) on startle potentiation or self-reported anxiety ($ps > .05$).

Discussion

This experiment provided clear evidence that anticipation of smoking is sufficient to reduce stress reactivity to discrete stressors for smokers in withdrawal. This finding emerged from a task that used potent, discrete stressors (i.e., threat of electric shock) to elicit stress reactivity that was assessed objectively by a well-validated measure of the CNS-modulated negative affective component of stress reactivity (i.e., startle potentiation). We assessed stress reactivity during three components of smoking as they would occur

[1] We tested for sex differences in preliminary analyses. However, no interactions involving sex were significant, so sex was removed from the final reported GLM.

[2] We removed from analysis an additional four deprived, two nondeprived, and two nonsmokers that were identified as regression outliers (i.e., studentized residual with Bonferroni corrected $p < .05$) in preliminary GLM analyses on startle potentiation.

Table 2
Startle Potentiation by Threat Type, Task Time, and Smoking Group

	Baseline			Anticipation			After consumption		
	Overall	U	P	Overall	U	P	Overall	U	P
Deprived smokers	20.0 (3.2)	19.9 (3.5)	20.2 (3.9)	9.1[a] (2.4)	8.2 (2.8)	10.0 (2.9)	6.1 (2.2)	7.2 (2.6)	5.0 (2.7)
Nondeprived smokers	16.0 (3.1)	14.7 (3.3)	17.4 (3.7)	15.5 (2.3)	13.2 (2.7)	17.7 (2.8)	15.0 (2.1)	11.4 (2.5)	18.5 (2.6)
Nonsmokers	14.9 (3.1)	14.7 (3.4)	15.2 (3.8)	14.9 (2.4)	14.8 (2.7)	14.9 (2.8)	9.6 (2.2)	8.5 (2.5)	10.7 (2.6)

Note. Means and standard errors for startle potentiation by threat type and task time adjusted for the interactive between-subject regressor for general startle reactivity (mean centered) for the entire final sample ($N = 108$). Overall = (unpredictable shock + predictable shock)/2; U = unpredictable shock; P = predictable shock.
[a] Denotes the significant effect ($p < .05$) of the planned contrast for Anticipation (anticipation of smoking vs. baseline).

in the real world (withdrawal, anticipation, smoking), which adds ecological validity to the current results. In addition, the effects of anticipation on startle potentiation were mirrored in supplemental analyses of participants' self-reported anxiety to the cues, which further validates these findings and rules out many methodological or measurement explanations for this effect. The lack of anticipation or consumption effects in both nondeprived and nonsmokers suggest the 24-hr deprivation manipulation drove these effects. Finally, anticipation of smoking dampened stress reactivity whether the threat of shock was predictable or unpredictable, which suggests that this effect is robust across various types of stressors that smokers may encounter in the real world. Although some research shows selective effects of drug consumption or withdrawal on response to unpredictable threat, the broad effects of anticipation seen here may implicate distinct neurologically and/or psychological mechanisms from previous translational work that did not measure anticipation (e.g., Hefner et al., 2013).

The effects of anticipation of drug use on stress reactivity can be compared and contrasted to cue reactivity research, which most often focuses on the emergence of cravings, changes in attention, and increased motivation to smoke in the presence of smoking cues (Carter & Tiffany, 2001). Much of the cue reactivity work uses smoking cues that are not directly predictive of cigarette consumption (i.e., pictures of other people smoking), and thus may not activate the cognitive and affective processes that occur in preparation for and anticipation of smoking (Juliano & Brandon, 2002; Levin, Rose, Behm, & Caskey, 1991; Perkins et al., 2008). Our paradigm is more comparable with cue-availability paradigms, in which smokers are informed that they will be able to smoke immediately after completing a task; however, these tasks also often utilize pictures in lieu of more powerful manipulations (Droungas, Ehrman, Childress, & O'Brien, 1995; McBride, Barrett, Kelly, Aw, & Dagher, 2006; but see Carter & Tiffany, 2001). In our study, we provided a powerful manipulation of smoking anticipation by having participants' own cigarettes remain in sight during the anticipation run of the task. Furthermore, most availability studies have not measured response to discrete stressors while participants are simultaneously anticipating drug use, as was done in this study.

The current findings suggest the need for more research in dissecting the cognitive-attentional and affective processes that lead up to a relapse (i.e., anticipation), and how such processes

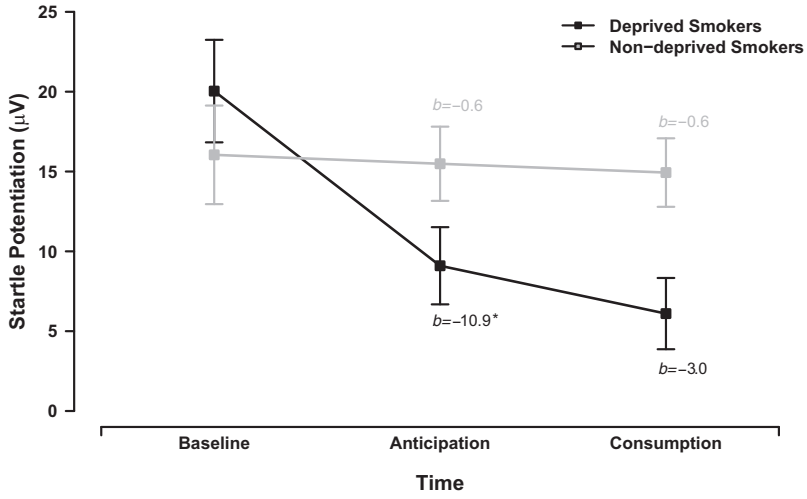

Figure 1. Startle potentiation by smoking group. Lines display the effects of anticipation and consumption on startle potentiation for deprived smokers (black) and nondeprived smokers (gray). Error bars indicate ± one standard error for point estimates of overall startle potentiation from the GLM. We report GLM coefficients for the simple effects of anticipation and consumption for each smoking group (*$p < .001$). GLM = general linear model.

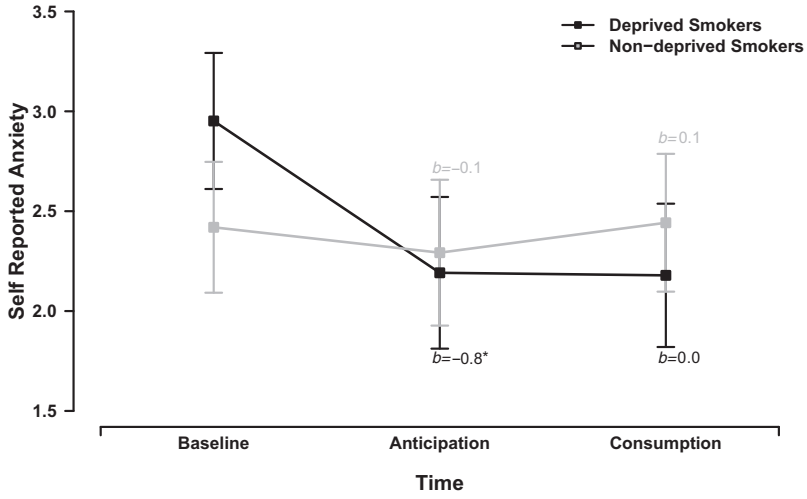

Figure 2. Self-reported anxiety by smoking group. Lines display the effects of anticipation and consumption on self-reported anxiety for deprived smokers (black) and nondeprived smokers (gray). Error bars indicate ± one standard error for point estimates of mean self-reported anxiety from the general linear model (GLM). We report GLM coefficients for the simple effects of anticipation and consumption for each smoking group ($^{*}p < .001$).

themselves may be rewarding by allowing a smoker to experience reduced stress and thus further perpetuated addiction. For instance, sensorimotor factors such as the sight, flavor, or inhalation of the smoke become secondary reinforces for smoking because of their repeated pairings with smoking itself (Caggiula et al., 2001; Perkins et al., 2001, 2008; Rose, Behm, Westman, & Johnson, 2000). Given that anticipation of smoking is frequently paired with smoking in a similar matter as sensorimotor cues, the rapid reduction in stress response triggered by anticipation suggests that anticipation of smoking may serve as another secondary reinforcer of smoking.

Distraction by the participant's cigarettes may also have contributed to our results. Recent research using cognitive tasks in availability paradigms have provided evidence that nicotine- deprived participants are more distracted by smoking-related cues (Juliano & Brandon, 1998; Robinson et al., 2014; but see Wertz & Sayette, 2001). Deprived smokers in our study may have been distracted by the cognitive processes involved in viewing their cigarettes or in thinking about smoking. Future research using noncigarette distractors, as well as cigarette cues that do not signal imminent smoking, in our paradigm may clarify the contribution of distraction to the stress dampening effects of anticipation seen in our study.

Smokers' stress reactivity was not reduced by smoking beyond the prior effect of anticipation. This result is somewhat in contrast to smokers' report that consumption of cigarettes reduces their stress (Parrott, 1999). However, this research may illustrate the inability of self-report to distinguish the processes involved in daily smoking. In other words, it may be that smokers associate the anticipation and planning with self-administration as a single event and are unable to differentiate the effects of the components (Baker, Japuntich, Hogle, McCarthy, & Curtin, 2006). Research with ecological momentary assessment during anticipation of smoking may be able to disentangle these processes in real world smoking (McCarthy, Piasecki, Fiore, & Baker, 2006; Piper et al., 2011). These findings are consistent with smoking-cue-reactivity studies that suggest that smoking cues alone have effects on smokers' mood when they believe they have consumed nicotine (Juliano & Brandon, 2002; Levin et al., 1991).

The present study narrowly focuses on the CNS component of the stress response. However, the stress response includes a complex interaction of CNS, peripheral nervous system, and hypothalamic-pituitary-adrenal (HPA) activations, which produce changes in affect, arousal, and attention (McEwen, 2001; McEwen, Eiland, Hunter, & Miller, 2012; Sapolsky, 2002, 2003; Segerstrom & Miller, 2004). Recent translational models of addiction heavily emphasize changes in components of the CNS (e.g., the extended amygdala) after chronic drug use (Koob & Volkow, 2010). These changes are believed to manifest as an increase in sensitivity to stressors, which may be reflected in humans by increases in negative affective response. In fact, work from our laboratory using similar methods as those used here has demonstrated increased sensitivity to various types of stressors in deprived smokers compared with nondeprived smokers (Hogle & Curtin, 2006; Hogle et al., 2010). It should be noted that we did not observe evidence of between-subject differences in baseline consistent with putative stress neuroadaptations in this study. However, this may not be too surprising, given that this study was not powered to detect such effects in only one run of the task (i.e., baseline). Nevertheless, our findings suggest that anticipation of smoking may have the potential to dampen the increased stress sensitivity previously seen in deprived smokers.

The affective and cognitive processes involved in drug use anticipation may represent an opportunity for intervention prior to drug use. For example, if anticipation of smoking holds equal or greater reinforcing value than smoking itself because of smokers' expectancies or implicit conditioning processes, education about such effects may help smokers to better understand their addiction. Or exposure therapy designed to weaken or break potential conditioned bonds between the cues, behaviors, and cognitions leading up to smoking, and the pharmacological effects thereof, may be an effective intervention. Future research is needed to better

understand mechanisms responsible for any role that anticipation has in maintaining addiction and to develop interventions that target this potential relapse process (e.g., McKee, Weinberger, Shi, Tetrault, & Coppola, 2012).

Smokers in the present study were allowed to smoke as much as they wanted during the consumption manipulation, yet they only smoked one cigarette, on average. This may raise concerns about whether there was sufficient dosing to reduce stress reactivity (Hogle & Curtin, 2006; Mueller, Mucha, & Pauli, 1998). Although the current research did not allow us to examine dose-specific effects, the ad lib smoking manipulation likely reflected the amount that smokers would smoke in reaction to some stressors in the real world. Future research could clarify dose effects by directly manipulating amount smoked or time allowed to smoke while comparing the effects of anticipation and actual smoking.

Future research could also assess what effect standard pharmacotherapies such as nicotine replacement (NRT) or varenicline have on stress reactivity in paradigms that measure smoking anticipation using human laboratory models that have been developed for screening interventions (McKee et al., 2012). Although NRT and varenicline have been shown to be effective in reducing postquit negative affect (Bolt, Piper, Theobald, & Baker, 2012; Cinciripini et al., 2013; Piper et al., 2008), it remains to be seen what effect they have on stress reactivity during anticipation of smoking. Of course, no current smoking cessation treatment is effective for all smokers (Fiore et al., 2008). Similarly, the effects seen here may not hold for all smokers. Smokers in this study were moderately dependent. Further work must be done to assess the effects of smoking anticipation on nondependent smokers (i.e., novice or nondaily smokers) or more dependent smokers, as they may exhibit even greater stress sensitivity. It is also important to explore these effects among smokers who may have increased sensitivity for other reasons (e.g., those with comorbid anxiety) who were excluded from this study.

In sum, the current research illustrates the importance of anticipation in reducing stress responses when a smoker is in withdrawal. These findings suggest that anticipation, rather than actual drug consumption, may drive the primary reinforcing effect of reduced stress responses in the context of drug use. The effects of anticipation should be further explored across different samples and using different levels of analysis of stress reactivity. It is possible that the stress-dampening effects of anticipation of use may generalize to other drug dependence. For example, alcohol consumption dampens startle potentiation to threat of shock in social drinkers (e.g., Bradford et al., 2013; Hefner et al., 2013; Moberg & Curtin, 2009), but it remains to be seen whether anticipation of drinking ameliorates stress responses in alcoholics. It is also remains to be seen whether the effects reported here manifest in indices of stress reactivity other than startle potentiation and self-report (e.g., direct measurement of HPA activity). Confirmation of this would inform research on the complex interplay between various subcomponents of the stress response system. More broadly, the anticipation effect seen for smoking could generalize to anticipation of other adaptive and maladaptive stress-relieving behaviors (e.g., eating, compulsions). If so, cognitive–behavioral treatments for various clinical disorders may benefit by incorporating anticipation of behavior as a target of intervention.

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