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A prospective study of stress and alcohol craving in heavy drinkers

Molly A. Tartter, Lara A. Ray *

Department of Psychology, University of California, Los Angeles, CA, USA

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ABSTRACT

Recent work has examined the relationship between stress and relapse to alcohol use in clinical populations. Few prospective studies, however, have examined stress as a precipitant of alcohol problems. The present study is a longitudinal examination of the role of stress reactivity and alcohol craving in the etiology of alcohol problems in a sample of 41 (mean age = 20.8), heavy-drinking, young adults. Participants completed a guided imagery exposure to stressful life events, followed by exposure to a neutral imagery control. Following the exposure, participants completed an alcohol cue exposure paradigm. Measures of negative mood (Profile of Mood States (POMS) depression/dejection scale), tension (POMS tension/anxiety scale) and alcohol craving (measured by the Alcohol Urge Questionnaire (AUQ)) were used as indicators of reactivity to stress and to alcohol cues. Polymorphisms of the corticotropin-releasing hormone binding protein (*CRH-BP*) gene and of the μ-opioid receptor (*OPRM1*) gene were examined as moderators of this relationship. Results revealed that stress-induced negative mood predicted negative consequences of drinking (scores on the Drinker's Inventory of Consequences (DrInC-2R)), whereas stress and cue-induced alcohol craving did not predict alcohol use or problems. Additionally, the *CRH-BP* genotype was found to moderate the relationship between stress-induced negative affect and the negative consequences of drinking. The current study supports and extends laboratory research describing phenotypes of stress-induced alcohol craving.

1. Introduction

Craving in response to alcohol cues or stress induction is an intermediate phenotype for alcoholism, and has been used in laboratory studies of addiction and relapse (Breese et al., 2005; Sinha, 2001, 2009). Dopamine signaling in the ventral tegmental area and forebrain is increased by alcohol use and provides positive reinforcement for drinking (Carrillo and Gonzales, 2011; Volkow and Fowler, 2000). Increased dopaminergic neurotransmission sensitizes the brain's reward system to the effects of alcohol, and is thought to promote drinking and its escalation (Verheul et al., 1999). Thus, craving for alcohol is hypothesized to result from the neuroadaptation of the dopaminergic reward system to repeated administration of alcohol (Robinson and Berridge, 2001).

Exposure to stress and priming doses of alcohol are commonly cited as reasons for relapse by patients (Adinoff et al., 1998; Breese et al., 2005; Cooney et al., 1997). Rohsenow and Monti (1999) found that the relationship between craving and relapse was entirely mediated by stress. Stress sensitivity is known to change in individuals who are alcohol dependent, and is a plausible mechanism of stress-induced relapse. Specifically, problem drinkers are thought to experience an inordinate amount of HPA axis activation as alcoholism worsens (Adinoff et al., 1998) and to have greater emotional arousal in response to stress, compared with healthy controls and social drinkers (Chaplin et al., 2010; Koob and Zorrilla, 2010; Sinha et al., 2009). In alcohol dependence, the hormonal markers of HPA axis activation are elevated both at the basal level and in response to alcohol withdrawal (Sinha et al., 2009). In addition, the ability of the HPA axis to react to stressors is compromised in addicted individuals (Costa et al., 1996; Inder et al., 1995).

Behavioral studies have found a relationship between stress-induced negative affect and craving in alcohol dependent individuals (Fox et al., 2008; Sinha et al., 2000, 2003). Reactivity to alcohol cues is increased by a history of detoxification, dependence on alcohol, and personality traits such as anger and anxiety (Litt et al., 2000). Stress-induction and alcohol cue exposure each produce negative affect and craving in alcohol dependent patients (Sinha, 2009). Stress induction produces greater negative emotionality, while an alcohol cue produces greater craving (Sinha, 2009). Research on the additive effects of stress and alcohol cues is mixed. While several studies have found that stress enhances the effects of cue-induced craving (Rubonis et al., 1994; Sinha and O'Malley, 1999), other studies have found no such additive effect (Litt et al., 2000; Ray, 2011).

Genetic variability is thought to play a large role in stress reactivity, and genetic predisposition to relapse may be moderated by stress (Blomeyer et al., 2008; Breese et al., 2005). Genetic variants of the

* Corresponding author at: Department of Psychology, University of California, Los Angeles, CA, 1285 Franz Hall, Box 951563, Los Angeles, CA 90095-1563, USA. Tel.: +1 301 794 5383; fax: +1 310 207 5895.
E-mail addresses: mtartter@ucla.edu (M.A. Tartter), lararay@psych.ucla.edu (L.A. Ray).

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corticotropin-releasing hormone (*CRHR1*) and its binding protein (*CRH-BP*) dictate the phenotypic expression of HPA axis responsivity. Recently, genetic variation in the CRH binding protein gene (*CRH-BP*) has been associated with alcoholism (Enoch et al., 2008) and the interaction between stressful life events and a polymorphism of *CRHR1* was found to predict heavy drinking in youth (Blomeyer et al., 2008).

The opioid system is examined here due to its involvement in the rewarding properties of alcohol (Chong et al., 2006; Kreek, 1996). In particular, the Asp40 variant at the A118G locus of the μ-opioid receptor gene (*OPRM1*) has been associated with dampened cortisol response, higher alcohol intake, and greater craving in reaction to stress (Kreek, 1996; Ray and Hutchison, 2004; Ray, 2011). The Asp40 variant also moderates subjective responses to alcohol and approach bias toward positive stimuli, including alcohol consumption (Oroszi, et al., 2009; Ray and Hutchison, 2007; Wiers et al., 2009). These findings indicate that *OPRM1* may be involved in the rewarding properties of alcohol, yet its role in stress-induced craving remains opaque. Several studies have found the Asp40 allele to selectively predict cue-induced craving and not stress-induced craving (Ray, 2011; van den Wildenberg et al., 2007). However, Pratt and Davidson (2009) found that the Asp40 allele predicted increases in craving *only* after stress induction. The current study examines the moderating effect of the A118G polymorphism on the relationship between cue- or stress-induced craving on subsequent alcohol use and alcohol-related problems.

While both stress and alcohol cues contribute to craving, they may function through different mechanisms. Thus, studies that combine stress and alcohol cues into a single manipulation (e.g., the same script) may be losing valuable information that can aid in characterizing these phenotypes. In order to examine the separate and combined effects of alcohol cues and stress on emotional reactivity and alcohol craving, we adapted a guided imagery paradigm from the procedures outlined by Sinha (2009). While a number of paradigms have been developed to examine stress in a laboratory setting (e.g., the Trier Social Stress Test, and the cold pressor test), they allow only for the study of specific types of stress. These paradigms do not reflect a range of life-like stressors, nor are they tailored to the individual. The adapted guided imagery paradigm allowed us to systematically expose participants to their own most recent, salient and stressful life events (Sinha, 2009). Guided imagery, like that used in the current study, has been shown to recreate the emotional experience of the stressor by activating memory networks (Lang et al., 1980). While previous work has separated alcohol cues and stress exposure into entirely different paradigms (Sinha, 2009; Fox et al., 2009), the present study sought to examine the ordered effects of stress and alcohol cue exposure. Thus, two imaginal exposure conditions were implemented. In the first, exposure to personalized stress imagery was followed by a water cue, and then an alcohol cue. In the second, participants were exposed to neutral imagery, followed by presentation of a water cue and then an alcohol cue. The presentation of stress exposure and neutral imagery exposure was counterbalanced and took place an hour apart. Alcohol cues were always presented last, so that no carry over effects would disrupt measurements of stress reactivity.

The present study examines craving for alcohol and stress-reactivity as predictors of future alcohol use and related problems. In addition, *OPRM1* and *CRH-BP* genotypes were examined as moderators of the direct effects of craving and emotional reactivity on drinking outcomes. Subjects were assessed at three time points. First, participants completed two counterbalanced exposures to stress and neutral imagery, each followed by an alcohol cue. Stress and alcohol cue exposure increased craving and negative mood over exposure to neutral imagery or a water cue (Ray, 2011). However, the effect of an alcohol cue was not additive with the effect of stress induction. Instead, alcohol cues produced greater negative mood, tension and craving after exposure to neutral imagery compared with stress imagery. Analyses at the baseline time point indicated that participants homozygous for the T allele of the *CRH-BP* gene (rs10055255) had higher craving, tension, and negative mood in response to stress induction than did A allele carriers (see Ray, 2011). Consistent with previous work, the *OPRM1* Asn40Asp SNP was found to predict greater alcohol craving in response to an alcohol cue after neutral imagery, but this effect was not amplified in the stress condition (van den Wildenberg et al., 2007; Wiers et al., 2009). Thus, the Asn40Asp SNP of *OPRM1* appears to confer predisposition to cue-induced craving, but not stress-induced craving.

Participants were contacted for an on-line follow-up at 6 and 12 months after evaluation in the laboratory. The present study examines the contribution of stress and craving measured experimentally to alcohol use and alcohol related problems across time. Data gathered from the 6 and 12 month follow ups was used to examine the following primary hypotheses: (1) Cue-induced craving for alcohol in the laboratory would predict an increase in alcohol consumption and problems related to alcohol use across the 12 months; (2) stress reactivity in the laboratory would predict increased alcohol consumption and drinking problems across time. In addition, the following secondary hypotheses were tested regarding the genetic variants of stress and reward systems: (1) the relationship between negative mood, tension and alcohol craving in response to stress would be moderated by *CRH-BP* genotype, such that individuals homozygous for the T allele of *CRH-BP* (rs10055255) with high stress reactivity would report heavier drinking and more alcohol-related problems across time; and (2) the relationship between craving for alcohol in response to an alcohol cue and alcohol use and problems would be moderated by *OPRM1* genotype, such that carriers of the Asp40 allele would report heavier alcohol use and problems in response to craving.

2. Method

2.1. Participants

Participants were 41, non treatment-seeking, heavy drinkers recruited for longitudinal data collection after participation in a laboratory study of stress and cue-induced craving for alcohol (for descriptive statistics and methods in the original study, see Ray, 2011). Participants met the following inclusion criteria: (1) age between 18 and 65; and (2) score of 8 or higher in the Alcohol Use Disorders Identification Test (AUDIT), which indicates a hazardous drinking pattern. Exclusion criteria for the laboratory study were: (1) currently receiving treatment for alcohol problems, a history of treatment in the 30 days before enrollment, or currently seeking treatment; (2) a lifetime diagnosis of schizophrenia, bipolar disorder, or psychotic disorder; and (3) current and regular, defined as once weekly, use of a psychoactive drug other than marijuana, as determined by self-report.

A total of 41 out of 60 participants consenting to be re-contacted (68.3%) completed the 6-month time point. Of these, 39 went on to complete the 12-month time point (data for 2 participants were lost due to problems with the online survey).

Demographic and clinical variables are presented in Table 1. Differences between follow-up completers and non-completers on baseline drinking and mood variables were observed. Completers had fewer alcohol-related problems at baseline ($p<.001$) and reported lower alcohol consumption in the 6 months prior to baseline assessment. Completers were marginally more likely to be female ($p=0.07$), but there were no differences in age, education or ethnicity (p-values >0.10). Self-reported monthly use of marijuana was assessed as a possible confound in the analyses, given the college-age sample. Marijuana use was numerically greater among non-completers ($M=21.95\pm36.98$) than completers ($M=8.95\pm17.87$), but not significantly so ($t(58)=1.46$, $p=0.16$), and there were no significant

Table 1
Demographic and clinical variables by gender.

	Female (25)	Male (16)	t/χ^2
Age, mean (SD)	19.88	19.95	−1.05
Ethnicity, *n*			7.55
Caucasian	8	21	
Asian	6	2	
Latino	2	1	
Af. American	0	1	
AUDIT Score, mean (SD)	11.81	15.75	−2.58*

*$p<.05$, **$p<.01$.

correlations between monthly marijuana use and the stress and cue-reactivity variables (*p*-values for linear regressions between 0.30 and 0.98). Gender was used as a covariate in the main analyses and did not significantly change any of the obtained results. The genotype groups within each SNP did not differ on age, gender, ethnicity or any of the predictor or outcome variables used in the study (*p*-values for χ^2 and ANOVAs >0.10). After subject attrition at the 6-month time point, the *CRH-BP* polymorphism remained in Hardy-Weinberg equilibrium ($\chi^2=1.20$, $p>0.05$), while the *OPRM1* polymorphism did not ($\chi^2=4.22$, $p<0.05$). Allele frequency distributions for the genes of interest are presented in Table 2.

2.2. Experimental procedures

Reactivity to stress, alcohol cue, or the combination of stress and alcohol cue, were measured at baseline using exposure to a tape-recorded script. Each participant completed two exposures: one to a personalized stress script, not related to drugs or alcohol, and one to neutral imagery; each was followed by a water and then an alcohol cue. The Profile of Mood States (POMS) and Alcohol Urge Questionnaire (AUQ; described below) are self-report measures used to assess negative mood, tension, and craving for alcohol after each exposure paradigm.

2.3. Follow-up procedures

All study procedures were approved by the Human Research Committee at the University of California, Los Angeles. Participants gave consent by phone, and all measures were collected via the online service, Survey Monkey. At each time point, participants were compensated with a gift certificate of their choice.

2.4. Experimental measures

2.4.1. Profile of mood states, short version (POMS; McNair et al., 1971)

The POMS is a 40-item measure of mood, widely used in human laboratory studies of addiction (Ray et al., 2009). Given the study aims, the tension/anxiety and depression/dejection subscales of the POMS were examined. These subscales, which we will refer to as "tension" and "negative mood", respectively, showed high internal consistency across experimental conditions. Cronbach's α for the tension subscale was between 0.84 and 0.91 and for the negative mood subscale: 0.88–0.93.

2.4.2. Alcohol Urge Questionnaire (AUQ; Bohn et al., 1995)

The AUQ is an 8-item scale on which subjects rate their craving for alcohol at the present moment. The observed reliability, measured by Cronbach's α, of the AUQ was high across administrations: $\alpha=0.92$ to 0.96. In a sample of 351 alcohol inpatients, the AUQ was validated by its relation to higher alcohol dependence severity, greater alcohol-related cognitive preoccupation, and shorter duration of abstinence (Bohn et al., 1995).

Table 2
Allele frequencies for genotypes of interest ($N=41$).

OPRM1 A118G genotype			*CRH-BP* rs.10055255 genotype		
AA	AG	GG	AA	AT	TT
33	6	2	8	24	9

2.5. Longitudinal measures

2.5.1. Modified Alcohol Consumption Questionnaire (Modified ACQ; Agrawal et al., 2009)

The ACQ was modified to encompass the four quantitative indices of alcohol use recommended by Agrawal et al. (2009), and to pertain only to the past 6 months of use, given the aims of the present study. The modified ACQ was given at 6 and 12 months follow-up time points, and collected the quantity × frequency of alcohol consumption during the past 6 months. The average number of drinks per day for an individual was derived from the ACQ and used as an outcome measure in analyses.

2.5.2. The Drinker Inventory of Consequences

(DrInc-2R; Tonigan and Miller, 2002) was used to assess the negative consequences of participants' drinking in the past 6 months. The DrInC-2R is a 50-item measure that provides a description of the number and frequency of drinking consequences for the past 6 months, and has been shown to be reliable and valid (Forcehimes et al., 2007). Cronbach's alpha was between 0.85 and 0.98. The total DrInC-2R score was used as an outcome measure.

2.6. Genotype selection and analysis

Based on the results of the experimental study (Ray, 2011), analysis of the OPRM1 gene was limited to the Asn40Asp SNP. Functional polymorphisms of the *CRH-BP* are not known. Several SNPs of interest were identified for examination in the experimental paradigm using the bioinformatics resources from the International HapMap Project, using a haplotype r^2 cutoff of 0.8 and a minor allele frequency (MAF) of 0.2. Results recommended rs10055255 and rs10062367. Rs10055255 was associated with stress reactivity in the experimental study and was chosen for examination at the follow up time points.

Saliva samples were collected under researcher observation for DNA analyses using Oragene saliva collection kits. Genotyping was performed at the UCLA Genotyping and Sequencing (GenoSeq) Core. Polymerase chain reaction (PCR) primers were labeled with fluorescent dye (6-FAM, VIC or NED), and PCR was performed on Applied Biosystems dual block PCR thermal cyclers. SNPs were run on an AB 7900HT Fast Real-Time PCR System and analyzed using the Sequence Detection Systems (SDS) software version 2.3. Each run included two positive control samples (individual 2 in CEPH family 1347; Coriell Institute). Genotypes were automatically scored by the allele calling software, and each genotype was verified by visual inspection. In process validation checks, the UCLA GenoSeq Core has average call, reproducibility, and concordance rates of 96%, 99.7%, and 99.8%, respectively. Quality values were computed for each genotype call in this sample, using a standard algorithm that combines various quality metrics. Genotype calls with a quality score of less than 95% were set to fail. Observed genotype call rates in this sample were 98.6% for the *OPRM1* SNP and 100% for the *CRH-BP* SNP.

2.7. Data analytic plan

Reactivity to stress was separated into the following predictors, using data from the POMS and AUQ: negative mood (POMS depression/dejection scale), tension (POMS tension/anxiety scale) and alcohol craving (from the AUQ). Cue-induced craving was also measured after each imagery condition (i.e., stress and neutral) using the AUQ. The three stress reactivity variables were obtained

by subtracting responses to the neutral condition, from responses to the stress condition. Two alcohol cue reactivity variables were obtained by subtracting craving following the water cue from craving following the alcohol cue in the stress and neutral conditions. Participants' tension and negative mood reactivity was measured as the difference between neutral and stress imagery.

t-Tests and chi-square tests in the PASW statistical package were used to examine differences between study completers and non-completers. Multilevel regression modeling was used in SAS statistical software to compare participants' trajectories of drinking, mood and negative consequences of drinking from baseline to 12 months (Singer, 1998). Time was assumed to be linear, and was centered at 0, and baseline measures of stress and cue-reactivity, as well as genotype were considered time invariant. Individual alcohol use and alcohol-related problems were allowed to vary across the 3 measured time points. These models were used to examine whether reactivity to stress and alcohol cue during the experimental portion of the study, genetic factors, and their interaction predicted differences in drinking at 6 and 12 months after the laboratory study, while controlling for baseline alcohol use.

3. Results

Descriptive statistics and bivariate correlations are presented in Table 3.

3.1. Stress reactivity as a predictor of alcohol use and problems

Stress reactivity was measured via negative mood, tension and alcohol craving. These reactivity variables were used to predict alcohol use and alcohol-related problems. There was a main effect of time in these models, indicating a decrease in drinking problems (DrInC-2R score) across time ($\beta=-4.97$, $p<0.01$). In addition, the interaction of negative mood×time predicted alcohol problems at 12 months ($\beta=8.93$, $p<0.01$), such that the slope of the relationship between negative mood and DrInC-2R score increased at each time point (baseline: $\beta=0.69$, n.s, at 6 months $\beta=4.87$, n.s, at 12 months $\beta=9.62$, $p<0.01$). Stress-induced craving did not predict alcohol use ($\beta=0.01$, n.s.) or alcohol problems across time ($\beta=0.00$, n.s.). Likewise, there was no effect of stress-induced tension on alcohol use ($\beta=-0.05$, n.s.) or alcohol problems across time ($\beta=1.26$, n.s.). Together, these results suggest that stress-induced negative mood, measured at baseline, significantly predicted an increase in DrInC-2R score over time, whereas stress-induced tension and alcohol craving did not.

3.2. Alcohol cue reactivity as a predictor of alcohol use and problems

Cue-induced craving following stress exposure did not significantly predict changes in alcohol use ($\beta=-0.02$, n.s.), or alcohol problems across time ($\beta=0.05$, n.s.). Likewise, cue-induced craving following neutral exposure was not a significant predictor of alcohol use ($\beta=0.16$, n.s.), or alcohol problems across time ($\beta=0.00$, n.s.). In sum, cue-induced craving measured in the laboratory was not associated with alcohol use and alcohol problems at 6 or 12-month follow-up.

3.3. Gene×stress and cue reactivity interactive effects on alcohol outcomes

3.3.1. CRH-BP

The corticotropin-releasing hormone binding protein SNP of interest (rs10055255) was coded so that T allele homozygotes were assigned a 1 and A allele carriers a 0. The predictors used in the model were *CRH-BP* genotype, negative mood reactivity to stress and their interaction across time (i.e., follow-up time points). An interaction effect was found for *CRH-BP*×time, such that T allele homozygotes had lower DrInC-2R scores at 6 months, compared with A allele carriers ($\beta=-18.86$, $p<0.05$). A 3-way interaction effect was found for *CRH-BP*×time× negative mood reactivity, where the relationship between negative mood and DrInC-2R score became stronger across time for T allele homozygotes. Within T allele homozygotes, the slope of the relationship between negative mood and DrInC-2R score increased significantly from 6 months to 12 months ($\beta=15.41$, $p=0.02$; *p*-values adjusted for multiple comparisons using the Sidak correction), and from baseline to 12 months ($\beta=23.51$, $p<0.001$). The increase from baseline to 6 months was not significant ($\beta=8.10$, *n.s.*; see Fig. 1). The slope of the relationship between negative mood and DrInC-2R score within A allele carriers was not significantly different from a slope of 0 at any of the time points in the study (baseline: $\beta=-1.40$, n.s.; 6 months: $\beta=3.04$, n.s.; 12 months: $\beta=-0.64$, n.s.; see Fig. 1). The interaction between *CRH-BP* genotype, negative mood reactivity to stress, and time did not predict alcohol use ($\beta=0.16$, n.s.). The interaction between *CRH-BP* genotype and tension reactivity to stress did not predict alcohol use across time ($\beta=0.15$, n.s.) or drinking problems across time ($\beta=1.83$, n.s.). The interaction of *CRH-BP* genotype and craving for alcohol in response to stress was not predictive of alcohol use ($\beta=-0.01$, n.s.) or problems across time ($\beta=-0.23$, n.s.).

3.3.2. OPRM1

The μ-opioid receptor polymorphism was coded for analysis using 0 to designate Asn40 (AA genotype) and 1 to designate Asp40 (G allele carriers). The interaction between *OPRM1* genotype and craving after alcohol cue in the stress condition did not predict alcohol use across time ($\beta=0.00$, n.s.) or alcohol problems across time ($\beta=0.70$, n.s.). The interaction between *OPRM1* genotype and craving in the neutral condition was not predictive of alcohol use ($\beta=0.03$, n.s.) or alcohol problems across time ($\beta=-0.84$, n.s.). In sum, *OPRM1* genotype did not moderate the effect of alcohol craving on patterns of consumption or problems due to alcohol use, as measured by the DrInC-2R.

Table 3
Correlation matrix of measured variables.

	1	2	3	4	5	6	7	8	9	10
1. Negative mood		0.74**	0.33*	0.21	−0.26	−0.17	−0.38	0.07	0.28	0.35*
2. Tension			0.15	0.19	−0.27	−0.26	−0.43*	−0.03	0.07	0.15
3. Cue-reactivity (neutral)				0.54**	−0.25	0.02	−0.26	0.06	−0.09	0.16
4. Cue-reactivity (stress)					−0.25	0.16	0.24	0.05	−0.05	0.06
5. Drinks per day (baseline)						−0.12	−0.19	−0.13	0.10	−0.02
6. Drinks per day (6 months)							0.69**	0.32*	0.31	0.33*
7. Drinks per day (12 months)								0.19	0.20	−0.01
8. DrInC-2R Score (baseline)									0.55**	0.54**
9. DrInC-2R score (6 months)										0.68**
10. DrInC-2R score (12 months)										
Means	0.90	1.32	5.98	3.73	2.45	1.87	1.70	24.76	21.41	18.74
(SD)	(0.79)	(1.02)	(7.55)	(6.06)	(2.08)	(1.41)	(1.57)	(9.07)	(13.20)	(18.47)

[a] *$p<.05$, **$p<.01$.

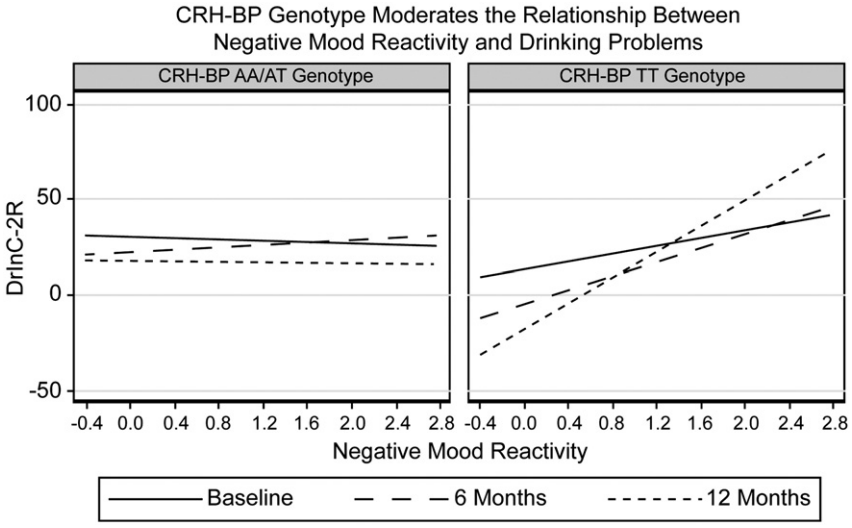

Fig. 1. The relationship between negative mood reactivity and DrInC-2R score is dependent upon CRH-BP genotype. Projected lines representing the relationship between negative mood reactivity and DrInC-2R score are shown at each time point by genotype. A allele carriers DrInC-2R score was not dependent on negative mood reactivity at baseline, 6 months, or 12 months. T allele homozygotes with higher negative mood reactivity showed an increase in drinking problems, measured by the DrInC-2R, across time points.

4. Discussion

We prospectively examined reactivity to stress and alcohol cues and their moderation by genotypes of interest in the CRH and opioid systems, as predictors of alcohol use and problems. Our primary hypothesis stated that increased craving for alcohol in response to stress and alcohol cues would predict alcohol use and alcohol-related problems longitudinally. Overall, a direct relationship between stress and cue-induced alcohol craving in the laboratory and subsequent alcohol use was not supported. Analyses revealed that stress-induced negative mood was associated with increased negative consequences of drinking across the yearlong follow-up, and that this relationship was stronger for participants with a common allelic variant of the *CRH-BP*. This is an important finding as it establishes the predictive utility of stress-reactivity in the laboratory for the development of alcohol problems in a community sample of at-risk drinkers. Further, it adds evidence for the contribution of genetic variation in the HPA axis to the development of alcohol problems over time (Enoch et al., 2008; Blomeyer et al., 2008).

Previous work examining the predictive value of stress and alcohol cue responses in the laboratory has focused on clinical samples (Cooney et al., 1997; Litt et al., 2000). Cooney et al. (1997) found that the combination of stress induction using imagery and the presentation of an alcohol cue was predictive of relapse in a dependent sample. The present study did not find a relationship between craving and alcohol use. Despite their heavy alcohol use, the present sample may be in a much earlier stage of the disorder than most clinical samples, based on their limited years of drinking. This may also explain the discrepancy with earlier findings that support craving as a predictor of use. Social and heavy drinkers' craving for alcohol increases equally in response to alcohol cues (Papachristou et al., 2011). Differential brain activity in alcoholics, as compared to social drinkers, however, may account for the difference in consumption (Myrick et al., 2004). Further, impulsivity has been shown to mediate the relationship between heavy drinking and increased craving in response to alcohol cues (Papachristou et al., 2011). Given their young age, and presumably brief drinking history (the present study did not take a drinking history beyond the past year) compared with clinical samples, study participants are not likely to have the neurobiological changes associated with chronic alcohol intake, and thus may be more akin to social drinkers than an alcohol dependent population in terms of neurobiology.

The present study drew on a sample of heavy-drinking young adults, many of whom were still enrolled in college. Recent studies have shown that alcohol use peaks during early college, as do alcohol-related problems (Jackson et al., 2008). Moreover, studies have shown that it is normative for most young people to reduce their drinking after they transition out of their student role, while a minority goes on to develop alcohol problems (Jackson et al., 2008). Research by Jackson et al. (2008) has identified four putative alcohol use trajectories in young adulthood: low heavy use, chronic heavy use, developmentally limited heavy use and late onset heavy use. In this sample, ratings of alcohol consumption and related problems declined, indicating that this group may discontinue heavy drinking.

Regarding the genetic hypotheses, we found that *CRH-BP* genotype moderated the relationship between negative mood reactivity to stress and the negative consequences of drinking. The moderation was such that T allele homozygotes showed a stronger relationship between negative mood and alcohol related problems at each successive time point. Within A allele carriers, the slope of the relationship between drinking problems and negative mood reactivity did not differ from 0 at any time point. These findings support the theory that *CRH-BP* is involved in the negative affective component of alcohol use disorders. Recent work has found relationships between two SNPs of the *CRH-BP* (rs 10474485 and rs1715747) as well as the μ-opioid receptor and depression symptoms in patients with alcohol dependence (Kertes et al., 2011). Interestingly, the authors did not find a direct connection between either of the genes and alcohol dependence symptoms. This work supports the present findings that *CRH-BP* is involved in moderating the relationship between negative mood and alcohol problems, but is not directly connected to alcohol use.

While preliminary, our results support the role of mood reactivity to stress as a moderator of the relationship between *CRH-BP* and negative consequences of drinking in a community sample. The combination of experimental manipulation with a longitudinal design is well suited to addressing the causal mechanisms underlying this association. These results suggest that further research on the role of stress reactivity, specifically mood, as a moderator of the relationship between the CRH system and alcohol problems is warranted.

The results have clinical relevance to early interventions for problematic alcohol use in college students. Our finding that negative mood and stress-reactivity contribute to the development of alcohol problems in young heavy drinkers is in line with previous clinical

findings regarding alcohol use in college populations. Kushner et al. (1999) found clinical levels of anxiety to be associated with drinking problems in college students, and Fager (2003) found poor coping skills to be predictive of binge drinking in this age group. A lack of stress-tolerance or coping skills components in campus alcohol interventions has been identified as an important area for further research (Fager and Melnyk, 2004). Additional research is needed to determine whether targeting stress-reactivity and negative mood states would be an effective addition to alcohol interventions for young hazardous drinkers.

We hypothesized that the *OPRM1* genotype would moderate the relationship between cue-induced craving and alcohol use and problems across time. Interactions between *OPRM1* and alcohol craving reactivity in response to alcohol cue were not predictive of drinking outcomes. While research on the Asn40Asp SNP of *OPRM1* supports its role in mechanisms of alcohol reward (Ramchandani et al., 2011), its predictive utility was not supported by the present analyses. In sum, our findings suggest that stress-reactivity, but not craving, is involved in the development and maintenance of alcohol-related problems in young, heavy drinkers, and is moderated by *CRH-BP* genotype.

Further work is needed to understand the role of *CRH-BP* genotype in moderating the relationship between negative mood and alcohol use. In concert with other neurotransmitters, *CRH-BP* genotype may represent a mechanism of negative reinforcement for alcohol use, despite negative psychosocial consequences (Koob, 2009). The interaction between CRH, the product of *CRH-BP*, and neuropeptide Y has been hypothesized to regulate emotional states (Heilig et al., 1994; Sajdyk et al., 2006). Specifically, CRH is thought to contribute to negative affectivity associated with alcohol abstinence (Leggio et al., 2011), while neuropeptide Y appears to regulate the anxiolytic effects of alcohol (Valdez and Koob, 2004). Future work examining the interactive effects of these genotypes may clarify the mechanism by which *CRH-BP* contributes to alcohol-related problems.

The current study has a number of strengths, including the opportunity for causal inference given the controlled experimental design, combined with longitudinal data collection. In addition, the examination of the predictive utility of laboratory-based phenotypes in the natural environment is a unique contribution to the behavioral pharmacology literature. Study limitations include the decrease in sample size over time, and differential attrition, as participants who dropped out reported higher alcohol consumption and more alcohol problems. Longitudinal studies following a larger sample over a longer period of time are needed to further elucidate the predictive utility of stress and cue reactivity on the development of alcohol problems. On balance, this study represents a much-needed first step in extending human laboratory research into longitudinal models.

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