Stress-Induced and Cue-Induced Craving for Alcohol in Heavy Drinkers: Preliminary Evidence of Genetic Moderation by the OPRM1 and CRH-BP Genes

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Background: Neurobiological theories of addiction have highlighted disruption in stress pathways as a central feature of addictive disorders, and pharmacological treatments targeting stress mechanisms hold great promise. This study examines genetic determinants of stress-induced and cue-induced craving in heavy drinkers by testing single-nucleotide polymorphisms (SNPs) of the corticotrophin-releasing hormone binding protein (CRH-BP) gene and the mu-opioid receptor (OPRM1) gene.

Methods: This study combines guided imagery stress exposure and in vivo alcohol cue exposure in a sample of 64 (23 women) non-treatment-seeking heavy drinkers.

Results: Analyses, uncorrected for multiple comparisons, revealed that a tag SNP of the CRH-BP gene (rs10055255) moderated stress-induced craving in this sample. The same SNP predicted greater affective responses to the stress manipulation, including greater levels of subjective tension and negative mood. The Asp40 allele of the OPRM1 was associated with greater cue-induced alcohol craving following the neutral imagery condition.

Conclusions: These initial results extend recent preclinical and clinical findings implicating the CRH-BP in stress-related alcoholism and confirm the role of the Asp40 allele of the OPRM1 gene in reward-driven alcohol phenotypes. Human laboratory models of stress and cue-induced craving may be useful in pharmacotherapy development targeting dysregulation of stress systems. Larger studies are needed to validate these preliminary findings, which should also be extended to clinical samples.

Key Words: Alcohol, Stress, Craving, Genetics, Corticotrophin-Releasing Hormone Binding Protein, Mu-Opioid Receptor.

THE ASSOCIATION BETWEEN stress and alcohol use has been well documented both in the preclinical (Koob and Kreek, 2007) and in the human (Uhart and Wand, 2009) literature. Neurobiological theories of addiction have highlighted disruption in stress pathways as a central feature of addictive disorders (Koob and Kreek, 2007), and studies have consistently supported an association between stress and relapse (Sinha, 2001). Acute social stress, under laboratory conditions, has been found to produce modest increases in alcohol use, even among social drinkers (de Wit et al., 2003). Human laboratory studies in alcoholism have allowed us to parse important markers of the disease risk and as such have contributed to our understanding of the biobehavioral bases of this disorder. Recently, these approaches have been leveraged to examine the genetic bases of alcohol use disorders (e.g., Mackillop et al., 2007; Ray and Hutchison, 2004) and to test the effects of medications for these disorders (e.g., Anton et al., 2004; Ray and Hutchison, 2007). Human laboratory studies of stress reactivity offer a unique opportunity to examine stress mechanisms underlying alcohol pathology and their genetic bases (Ray et al., 2010a). However, to date, these methods have not been sufficiently leveraged as a tool for behavioral genetics research in alcoholism.

A promising human laboratory model of stress and addiction consists of guided imagined exposure to stressful events (Sinha, 2009). This paradigm is based largely on Lang's emotional imagery methodology (Lang, 1979; Lang et al., 1980) and consists of obtaining information about recent stressful, neutral, and alcohol/drug-related events in participants' lives and using that information to develop individualized scripts that can elicit stress response under laboratory conditions. These methods have proven to be valid, reliable, and useful in advancing research on stress and addiction (Sinha, 2008), particularly in explaining relapse (Sinha, 2007). The present study combines the imagined stress exposure paradigm with in vivo cue-reactivity and extends the literature on these phenotypes by first, providing a unique combination of well-established laboratory phenotypes, and second, examining candidate genes that may moderate stress-induced and cue-induced craving for alcohol.

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It has been argued that as addiction progresses, the avoidance of unpleasant and stressful emotional states becomes central to the maintenance of the disorder (Koob, 2003). Efforts to understand neuroadaptation of stress pathways have focused on the overactivation of the corticotrophin-releasing hormone (CRH), also termed corticotrophin-releasing factor (CRF), as a key mediator of stress sensitivity and negative affect (Heilig and Koob, 2007). Recent preclinical data indicated that blocking hyperactive signaling at CRH receptors reduces the risk of relapse by blocking stress, but not alcohol cues associated with relapse (Heilig and Koob, 2007). This work highlights the need to effectively probe the CRH system for addiction vulnerability, including genetic factors. To that end, this study examines genetic variation in the CRH-binding protein (CRH-BP), which has been shown to modulate the effects of CRH on stress-induced relapse in preclinical models (Wang et al., 2005, 2007). A recent study with clinical samples suggested that genetic variation in CRH-BP was significantly associated with alcoholism in Caucasian samples and anxiety disorders in Plains Indians (Enoch et al., 2008). Moreover, a pharmacogenetic analysis of the STAR*D study dataset revealed that CRH-BP polymorphisms were associated with better clinical response to citalopram for depression, as evidenced by both remission and reduction in depressive symptoms (Binder et al., 2010). Interestingly, this association was stronger among patients with features of anxious depression, suggesting a role for anxiety and stress phenotypes in this study. In light of the existing literature, the present study investigates whether this candidate gene moderates stress- and cue-induced alcohol craving in a sample of heavy drinkers.

In addition, this study examines a functional single-nucleotide polymorphism in the mu-opioid receptor (OPRM1) gene [Asn40Asp single-nucleotide polymorphisms (SNP)]. This SNP was selected based on previous reports by our group and others, suggesting that this polymorphism is associated with subjective responses to alcohol, including craving (Filbey et al., 2008; Ray and Hutchison, 2004; Ray et al., 2010b; van den Wildenberg et al., 2007) and more recently, stress-induced drinking (Pratt and Davidson, 2009). The opioidergic system plays an important role in stress response via regulation of the hypothalamic-pituitary-adrenal (HPA)-axis. Specifically, CRH neurons in the hypothalamus are under tonic inhibition by neurons containing β-endorphin; hence, the OPRM1 gene was advanced as a candidate gene for stress-induced craving in this study. Unlike the CRH-BP gene for which no functional SNPs have been identified to date, the Asp40 allele has been associated with higher binding affinity for beta endorphin (Bond et al., 1998) and decreased mRNA yield (Zhang et al., 2005). Carriers of the Asp40 allele have shown greater cortisol response to the opioid antagonist naloxone (Hernandez-Avila et al., 2003; Wand et al., 2002). Consistent with studies demonstrating greater hormonal and psychological stress response among Asp40 carriers, it is hypothesized that carriers of the Asp40 allele of the OPRM1 gene will display greater stress- and cue-induced craving. Likewise, based on clinical and preclinical findings, it is hypothesized that genetic variation in the CRH-BP gene, captured via tag SNPs, will be associated with stress and cue-induced craving in this sample of at-risk drinkers. This study seeks to extend preclinical and clinical findings implicating stress system dysregulation in alcoholism by leveraging well-established mechanistic probes of stress and cue-reactivity to elucidate the role of the ORPM1 and CRH-BP candidate genes in these phenotypes.

PARTICIPANTS AND METHODS

Participants

Participants (n = 64, 23 women) were non-treatment-seeking heavy drinkers who met the following inclusion criteria: (i) age between 18 and 65 and (ii) score of 8 or higher in the Alcohol Use Disorders Identification Test (AUDIT; Allen et al., 1997), indicating a hazardous drinking pattern. Exclusion criteria were (i) currently receiving treatment for alcohol problems, a history of treatment in the 30 days before enrollment, or currently seeking treatment; (ii) a lifetime diagnosis of schizophrenia, bipolar disorder, or any psychotic disorder; (iii) current and regular (defined as once weekly) use of psychoactive drug, other than marijuana, as determined by self-report.

Procedures

All study procedures were approved by the Human Research Committee at the University of California, Los Angeles, and all participants provided written informed consent after receiving a full explanation of the study. Interested individuals called the laboratory and completed a telephone screen for eligibility, including the AUDIT. Following telephone screening procedures, participants completed 2 in person testing sessions (scheduled 1 week apart). At the beginning of each laboratory session, participants were breathalyzed and required to have a breath alcohol concentration (BAC) equal to 0.000 in order to complete each session.

During Session 1, participants provided written informed consent, completed individual differences measures, received standardized relaxation training and imaginal exposure training (designed to enhance their ability to complete the guided imagery protocol), and provided detailed descriptions of recent stressful and neutral life events. This information was used to generate tape-recorded personalized scripts for the neutral and stressful experimental conditions, following well-established procedures (Sinha, 2009; Sinha et al., 1992, 2000). Participants were asked to identify and describe recent stressful experiences and to rate them on a 0 to 10 Likert scale, where 10 is the most stressful. Only stressful events rated ≥8 were used in script development. Stressful events that were resolved were not used in script development to ensure the salience of the stimuli presented. Data on physical feelings and sensations associated with the stressful (and neutral) events were also collected for the purpose of script development. All scripts were evaluated by the author for stressful and neutral content prior to implementation.

During Session 2, participants completed 2 guided imagined exposures (Stress and Neutral), each followed by an alcohol cue exposure. The experimental conditions (Stress and Neutral) were conducted in randomized and counterbalanced fashion. Each exposure consisted of 5-minute tape-recorded scripts recounting stressful (or neutral) recent events in the participants' lives, including cognitions and physical feelings (of stress or neutral/relaxed) associated with their reports of the experience. Stress and Neutral imagery exposure (counterbalanced) took place within a single session and were separated by 1 hour to avoid carryover effects.

Each guided imagery condition was followed by cue exposure (CE), following well-established procedures (Monti et al., 1987,

2001). Participants were systematically exposed to water and alcohol beverages. Order of alcohol and water stimuli was not counterbalanced because of carryover effects that are known to occur (Monti et al., 1987) and that would interfere with the determination of cue-reactivity. The water trial provides a baseline that controls for all aspects of stimuli except the nature of the beverage. Observation through a 1-way mirror was used to ensure compliance.

Measures

Demographic information was collected, including age, gender, ethnicity, education, and employment. Data regarding quantity and frequency of drinking were collected using the Time Line Follow Back (TLFB), a calendar-assisted interview of alcohol use over the past 30 days (Sobell et al., 1996). During Session 2, the following measures of mood and urge to drink were administered repeatedly at baseline, postimagery, and postcue exposure (Water and Alcohol) in both Stress and Neutral experimental conditions. These measures of mood and alcohol craving represent the primary dependent measures in this study.

Profile of Mood States, Short Version (McNair et al., 1971). The Profile of Mood States (POMS) is a 40-item measure of mood, widely used in human laboratory studies of addiction (Ray et al., 2009). Given the study aims, Tension and Negative mood were the only subscales of the POMS examined. Each subscale score is composed of 10 items rated on a 0 to 5 Likert scale. These 2 subscales showed high internal consistency across experimental conditions with Cronbach α between 0.84 to 0.91 (Tension) and 0.88 to 0.93 (Negative Mood).

Alcohol Urge Questionnaire. The Alcohol Urge Questionnaire (AUQ) is an 8-item scale in which subjects rate their craving for alcohol at the present moment. The AUQ is appropriate for repeated administration within studies of state levels of urge to drink (Bohn et al., 1995; MacKillop, 2006). The observed reliability of the AUQ was high across administrations, α 0.92 to 0.96.

Genotype Selection

Consistent with the study hypotheses, analysis of the OPRM1 gene prioritized the Asn40Asp SNP, given its known functional significance. In order to examine the role of the CRH-BP gene, for which functional polymorphisms are not known, our approach was to use the bioinformatics resources from the International HapMap Project to identify tag SNPs (tSNPs) for that gene. The parameters for our search were haplotype r^2 cutoff = 0.8 and a minor allele frequency (MAF) of 0.2. Results recommended the following 2 tSNPs: rs10055255 and rs10062367. This approach assumes that as the majority of SNPs are not functional, associations may be as a result of linkage disequilibrium (LD) with a risk-increasing allele. Therefore, even at very high LD (range = 0–1, where 1 represents total LD), differences in allele frequency between the candidate SNP or tSNP and the risk-increasing locus can have a significant impact on the power to detect associations. Figure 1 displays an LD plot for individuals of European Ancestry generated from publicly available Hapmap (phase III) data using the software program Haploview v4.2 (Barrett et al., 2005).

Genotyping

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 (Carlsbad, CA) dual block PCR thermal cyclers.

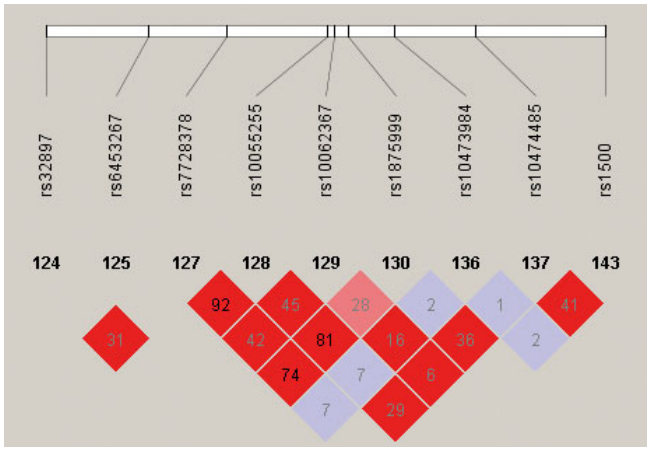

Fig. 1. Linkage disequilibrium plot from Haploview 4.2 for based on Hapmap (phase II) samples of individuals of European Ancestry for the 2 corticotrophin-releasing hormone binding protein single-nucleotide polymorphisms (SNPs) evaluated in this study (rs10055255 and rs10062367), SNPs evaluated by Enoch and colleagues (2008) (rs32897, rs6453267, rs7728378, rs18785999, rs10474485, and rs1500), and SNPS evaluated by Binder and colleagues (2010) (rs10473984, rs10055255, and rs10474485). The numbers represent R^2 values.

SNPs were run on an AB 7900HT Fast Real-Time PCR System and analyzed using the Sequence Detection Systems software version 2.3. Each run included 2 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 $<95\%$ were set to fail. Observed genotype call rates in this sample were 98.6% for the OPRM1 SNP and 100% for each of the CRH-BP SNPs.

Power Analysis

Power analysis was conducted using the continuous outcome design option in Quanto (Gauderman, 2002a,b, 2003). Tests estimated the power to detect genetic effects for a continuous outcome in a sample of 64 unrelated individuals. The following parameters were used in this power analysis: (i) MAF of 0.14, which is the observed MAF in this sample. However, we note that MAF varies widely within Caucasian groups (reported MAF range of 0.025–0.155) (Arias et al., 2006) and (ii) dominant gene action. Statistical power was estimated at 2 alpha levels, 0.05 and 0.01, in order to assess the changes in statistical power resulting from possible corrections for Type I error. At an alpha level of 0.05, a dominant locus accounting for 9% or more of the overall variance would be detectable with approximately 80% power. Conversely, at an alpha level of 0.01, the smallest genetic effect size detectable with adequate (80%) power would account for 14% of the variance in a given phenotype, which is equivalent to a large effect size. As a reference, a small effect size corresponds to $R^2 = 0.01$, medium effect size $R^2 = 0.06$, and large effect size $R^2 = 0.14$ (Cohen, 1988, 1992). The partial η^2 provided for the analyses below can be interpreted as R^2 estimates, as they index the proportion of the variance in the dependent variable explained by a given predictor (independent) variable.

Data Analysis

The following data analytic procedures were used to address the study objectives. A series of mixed models analysis of variance was

conducted in which Imagery (Stress vs. Neutral) and Trial (Before and After Imagery) were within-subject factors, Genotype (OPRM1 and CRH-BP, each SNP tested separately) was a between-subjects factor, and scores on mood and alcohol craving were the dependent measures. Identical analyses were conducted examining Cue (Water vs. Alcohol). Corrections for Type I error were considered but ultimately rejected on the basis of the following considerations. First, correction for Type I error would result in a significant loss of statistical power for the genetic analyses, which is a significant issue as detailed previously. Second, Type I error needs to be considered for each hypothesis separately, not for the number of variables in the whole set of analyses reported (Dar et al., 1994). In the present study, it is hypothesized that the polymorphisms of the OPRM1 and CRH-BP genes will moderate stress-induced and cue-induced craving for alcohol; hence, only 4 hypotheses are being tested.

Baseline Differences

All variables were found to exhibit an adequately normal distribution suggesting that no transformations were warranted. The genotype groups within each SNP (CRH-BP rs10055255, CRH-BP rs10062367, and OPRM1 Asn40Asp SNP) did not differ on sociodemographic (i.e., gender, age, ethnicity) or drinking variables (i.e., AUDIT score, drinking quantity, and drinking frequency in the past year), all p-values > 0.10. Given the potential for population difference to confound the effects of genotype, all analyses were repeated including only Caucasian subjects, the most common ethnic group.

RESULTS

Sample Characteristics

A total of 64 participants (23 women) completed this study. Sample demographic and alcohol use characteristics by gender are presented in Table 1.

Allele frequencies for the OPRM1 A118G SNP, CRH-BP rs10055255, and CRH-BP rs10062367 are presented in Table 2. All 3 SNPs were in conformity with Hardy–Weinberg Equilibrium, $\chi^2(1) = 3.22$, 0.00, and 0.01, respectively, p-values = 0.07, 1.0, and 0.99. Consistent with previous studies, analyses of the OPRM1 SNP compared Asp40 carriers to Asn40 homozygotes. In order to increase statistical power to detect genetic effects, the CRH-BP rs10055255 A-allele carriers (AA and AT) were combined and compared to T-allele homozygotes (TT). This is consistent with the observed pattern of genetic dominance by the A-allele on the dependent variables of interest. CRH-BP rs10062367 analyses compared A-allele carriers (AA/AG) to G-allele homozygotes (GG).

Table 1. Demographic and Alcohol Use Characteristics by Gender

	Female (23)	Male (n = 41)	t/χ^2	p
Age, mean (SD)	20.3 (1.5)	21.0 (3.1)	−1.33	0.19
Ethnicity, *n*				
Caucasian	11	36	14.45	<0.05
Asian	8	3		
Latino	4	1		
African American	0	1		
AUDIT Score, mean (SD)	12.9 (3.8)	17.1 (5.8)	−3.51	<0.001
Drinks per episode, mean (SD)[a]	4.8 (2.2)	6.5 (2.9)	−2.34	<0.05
Drinking days, mean (SD)[a]	7.8 (5.2)	13.8 (7.4)	−3.43	<0.01

[a]Drinking data obtained from the 30-day Time Line Follow Back (TLFB) interview.

AUDIT, Alcohol Use Disorders Identification Test.

Table 2. Allele Frequencies for Genotypes of Interest (total n = 64)

OPRM1 A118G			CRH-BP rs10055255			CRH-BP rs10062367		
AA	AG	GG	AA	AT	TT	GG	GA	AA
49	12	3	16	32	16	39	21	3

CRH-BP, corticotrophin-releasing hormone binding protein; OPRM1, mu-opioid receptor.

Combining Stress and Alcohol Cues

Results of manipulation checks comparing baseline to postimagery mood and craving ratings revealed that the Stress condition produced greater levels of alcohol craving ($F_{1,63} = 29.98$; $p < 0.0001$; partial $\eta^2 = 0.32$), tension ($F_{1,63} = 105.65$; $p < 0.0001$; partial $\eta^2 = 0.63$), and negative mood ($F_{1,63} = 94.28$; $p < 0.0001$; partial $\eta^2 = 0.60$), when compared to the Neutral condition. Given that the Imagery condition was a within-subject factor, analyses examined order effects. Results supported the efficacy of the randomization and counterbalancing of the imagery conditions and found no evidence of order effects on any of the dependent measures of interest, p-values > 0.10.

There was a significant Stress × Sex × Trial effect on craving ($F_{1,62} = 4.10$; $p < 0.05$; partial $\eta^2 = 0.06$), tension ($F_{1,62} = 8.31$; $p < 0.01$; partial $\eta^2 = 0.12$), and negative mood ($F_{1,62} = 5.47$; $p < 0.05$; partial $\eta^2 = 0.08$), such that women reported greater stress-induced urge to drink, tension, and negative mood, when compared to men. However, there was no gender effect on cue-reactivity ($F_{1,62} = 2.93$; $p = 0.09$; partial $\eta^2 = 0.04$), Importantly, controlling for gender did not significantly alter any of the results reported herein.

Analyses of the cue-reactivity paradigm indicated that the presentation of alcohol cues increased alcohol craving ($F_{1,63} = 53.50$; $p < 0.0001$; partial $\eta^2 = 0.46$), and negative mood ($F_{1,63} = 27.53$; $p < 0.0001$; partial $\eta^2 = 0.30$), across both Stress and Neutral imagery conditions. Alcohol cues had no main effect on ratings of tension ($F_{1,63} = 1.02$; $p = 0.32$; partial $\eta^2 = 0.02$). Interestingly, the effects of alcohol cues were not multiplicative to the effects of the stress manipulation. To the contrary, stress condition moderated the relationship between alcohol cues and ratings of alcohol craving and mood such that alcohol cues produced greater increases in craving ($F_{1,63} = 15.11$; $p < 0.001$; partial $\eta^2 = 0.19$), tension ($F_{1,63} = 47.06$; $p < 0.0001$; partial $\eta^2 = 0.43$), and negative mood ($F_{1,63} = 44.24$; $p < 0.0001$; partial $\eta^2 = 0.41$), after Neutral imagery when compared to Stress imagery. As shown in Fig. 2, the alcohol cue exposure caused the experimental

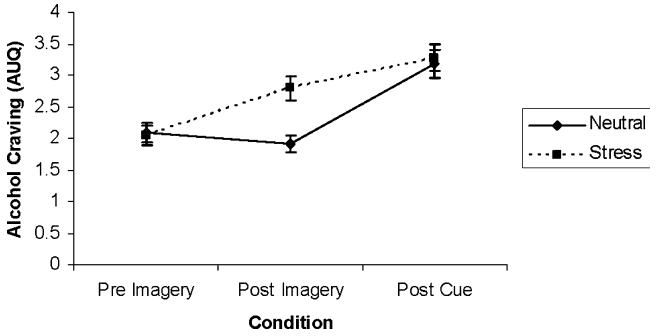

Fig. 2. Mean (±SEM) alcohol craving rating at baseline, postimagery, and postalcohol cue in both neutral and stress conditions.

conditions to be more similar in alcohol craving (as well as on mood ratings), suggesting that higher craving for alcohol was reached via either stress manipulation or via alcohol cue exposure, yet the effect of stress plus cues was not multiplicative. In other words, stress attenuated responsivity to alcohol cues. Results from this novel combination of laboratory paradigms suggest that stress-induced and cue-induced craving may be dissociated. As such, subsequent analyses of genetic effects will examine the genetic moderators by testing stress-induced alcohol craving and cue-induced alcohol craving separately.

Corticotrophin-Releasing Hormone Binding Protein Findings

Corticotrophin-Releasing Hormone Binding Protein rs10055255. Analyses revealed a significant Genotype × Stress Condition × Trial interaction suggesting that the greatest increase in alcohol craving occurred poststress imagery ($F_{1,62} = 5.41$; $p < 0.05$; partial $\eta^2 = 0.08$). Planned comparisons indicated that while Genotype did not moderate changes in alcohol craving following Neutral imagery ($F_{1,62} = 1.60$; $p = 0.21$; partial $\eta^2 = 0.03$), there was a significant Genotype effect on alcohol craving following Stress imagery ($F_{1,62} = 3.99$; $p = 0.05$; partial $\eta^2 = 0.06$) (Fig. 3*A*). In addition, there was a significant Genotype × Stress Condition interaction ($F_{1,62} = 4.83$; $p < 0.05$; partial $\eta^2 = 0.07$), such that homozygotes for the T allele of rs10055255 reported greater alcohol craving during the Stress condition, but not Neutral condition, when compared to A-allele carriers.

Consistent with the findings of a genotype effect for rs10055255 on stress-induced craving for alcohol, analyses revealed a significant Genotype × Stress Condition × Trial interaction ($F_{1,62} = 4.06$; $p < 0.05$; partial $\eta^2 = 0.06$), such that T-allele homozygotes reported greater stress-induced tension, when compared to A-allele carriers. These results were supported by planned comparisons indicating that CRH-BP genotype moderated tension ratings after Stress imagery ($F_{1,62} = 4.57$; $p < 0.05$; partial $\eta^2 = 0.07$), but had no effect during the Neutral imagery condition ($F_{1,62} = 0.67$; $p = 0.42$; partial $\eta^2 = 0.01$) (Fig. 3*B*).

There was also a significant Genotype × Stress Condition × Trial interaction regarding negative mood ($F_{1,62} = 10.13$; $p < 0.01$; partial $\eta^2 = 0.14$), indicating that T-allele homozygotes reported greater negative mood following Stress imagery ($F_{1,62} = 9.04$; $p < 0.01$; partial $\eta^2 = 0.13$), but not after Neutral imagery ($F_{1,62} = 2.17$; $p = 0.15$; partial $\eta^2 = 0.03$), compared to A-allele carriers (Fig. 3*C*).

There was no Genotype × Stress Condition × Trial interaction regarding cue-induced alcohol craving ($F_{1,62} = 0.33$; $p = 0.57$; partial $\eta^2 = 0.01$). Together, these results revealed

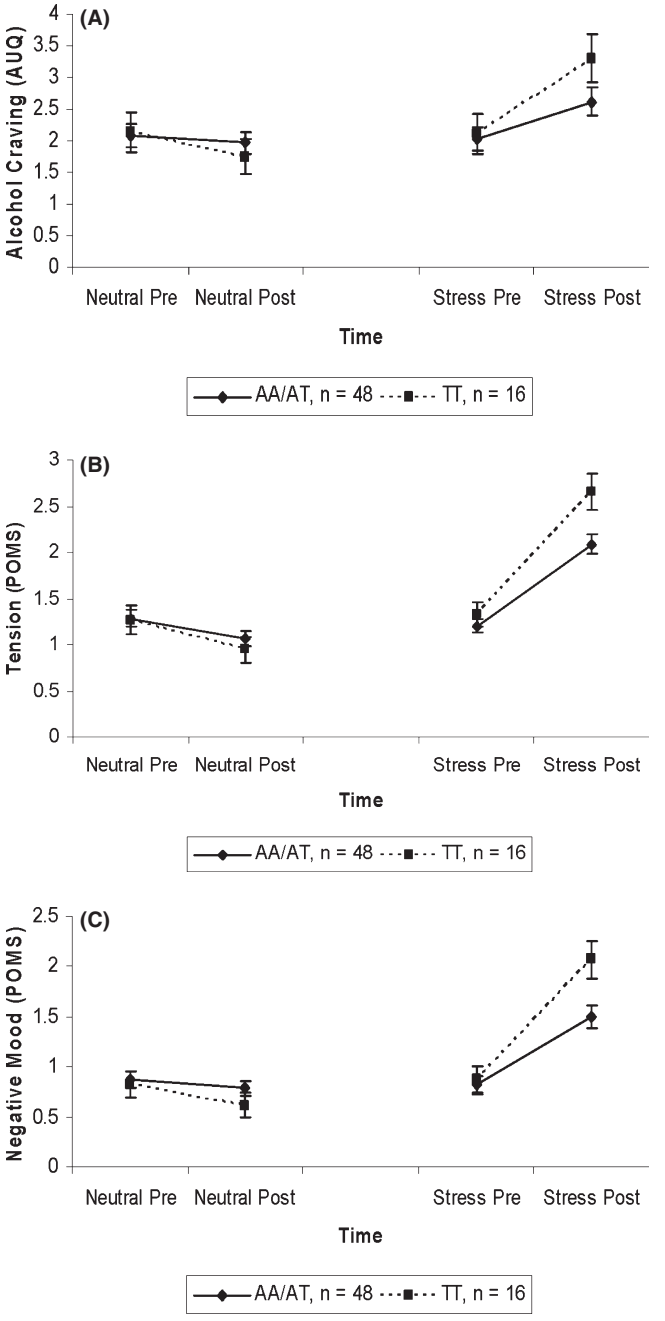

Fig. 3. Mean (±SEM) rating at baseline and postimagery for both neutral and stress conditions by corticotrophin-releasing hormone binding protein rs10055255 genotype for alcohol craving (**A**), tension (**B**), and negative mood (**C**). Analyses compared A-allele carriers (AA/AT) versus T-allele homozygotes (TT).

that compared to A-allele carriers, T-allele homozygotes for rs10055255 reported greater stress-induced alcohol craving, tension, and negative mood during this laboratory-based stress manipulation.

Corticotrophin-Releasing Hormone Binding Protein rs10062367. Analyses revealed no significant Genotype × Stress Condition × Trial interaction with regard to stress-induced craving ($F_{1,62} = 2.00$; $p = 0.16$; partial $\eta^2 = 0.03$), stress-induced tension ratings ($F_{1,62} = 3.17$; $p = 0.08$; partial $\eta^2 = 0.05$), or stress-induced negative mood ($F_{1,62} = 2.69$; $p = 0.11$; partial $\eta^2 = 0.04$). In addition, there was no genotype effect on cue-induced craving ($F_{1,62} = 0.01$; $p = 0.93$; partial $\eta^2 = 0.00$).

Mu-Opioid Receptor Findings

There was no Genotype × Stress Condition × Trial interaction with regard to stress-induced craving ($F_{1,62} = 2.83$; $p = 0.10$; partial $\eta^2 = 0.04$), stress-induced tension ($F_{1,62} = 0.21$; $p = 0.65$; partial $\eta^2 = 0.00$), or stress-induced negative mood ($F_{1,62} = 0.18$; $p = 0.68$; partial $\eta^2 = 0.00$). Analysis of cue-induced craving revealed a Genotype × Stress Condition interaction ($F_{1,62} = 4.50$; $p < 0.05$; partial $\eta^2 = 0.07$), suggesting that Asp40 carriers reported greater cue-induced craving during the neutral condition, when compared to the stress condition. In addition, there was a significant Genotype × Trial interaction ($F_{1,62} = 6.39$; $p < 0.05$; partial $\eta^2 = 0.10$), such that carriers of the Asp40 allele reported greater alcohol craving, upon exposure to alcohol cues, than Asn40 homozygotes (Fig. 4).

Probing for Population Stratification Effects

In light of the potential for population differences in allele frequencies to confound the genotype effects, analyses were repeated including only Caucasian individuals ($n = 47$, 73.5% of the total sample of 64 study completers). The results of such analysis supported the overall pattern of effects seen in the larger sample, yet statistical significance reflected reduced statistical power associated with the smaller sample size. In order to provide an unbiased comparison, estimates of effect sizes (partial η^2) were obtained and compared for the full sample ($n = 64$) versus the Caucasian-only sample ($n = 47$) on the significant genetic effects reported previously. Effect sizes for the CRH-BP gene (rs10055255) on stress-induced craving, tension, and negative mood in the full sample were as follows: partial $\eta^2 = 0.08, 0.06, 0.13$, respectively. In the Caucasian sample only, effect sizes were relatively unchanged, partial $\eta^2 = 0.07, 0.05, 0.22$, respectively. For the OPRM1 gene, the Genotype × Stress Condition effect remained relatively unchanged (partial $\eta^2 = 0.07$ for full sample; 0.05 for Caucasian-only sample), suggesting higher alcohol craving for Asp40 carriers on the Neutral condition, when compared to Stress. However, the Genotype × Trial interaction suggesting greater cue-reactivity by Asp40 allele carriers was dampened in the Caucasian-only sample (partial $\eta^2 = 0.10$ for full sample; 0.02 for Caucasian-only sample).

As an alternative approach to probing for population stratification, we controlled for ethnicity in all significant genetic models and then re-estimating effect sizes within those models. Doing so revealed that all of the original effect sizes remained unchanged. In summary, with the exception of the OPRM1 effect on cue-reactivity that remained significant only in the neutral condition, all genetic effects were supported by follow-up analyses probing for population stratification as a confound.

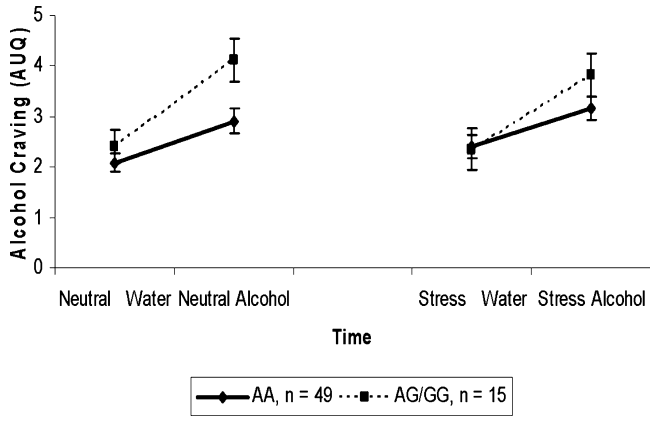

Fig. 4. Mean (±SEM) alcohol craving rating after water cue and alcohol cue in both neutral and stress conditions by OPRM1 Asn40Asp genotype.

DISCUSSION

Behaviorally, the present findings demonstrate dissociation between stress-induced and cue-induced craving for alcohol among heavy drinkers. Individuals reached higher levels of subjective alcohol craving upon exposure to alcohol cues and those for whom the cues were preceded by stress-induction, reported their craving to be at an intermediate point between baseline and postcue exposure. Conversely, following the neutral imagery condition, subjective craving did not change until cues were presented. This is different from models in which stress and cues are combined into a single script (Sinha et al., 2009) and suggests that in vivo cue exposure may be more potent leading to the observed dissociation between stress-induced and cue-induced craving. This finding is also consistent with earlier work suggesting that the presence of negative mood alone, following a mood induction, was sufficient to elicit alcohol craving regardless of cue exposure (Litt et al., 1990) and was predictive of relapse (Cooney et al., 1997). Studies counterbalancing stress and alcohol cues are needed to confirm the observed stress and cues dissociation.

The observed dissociation has implications for efforts to define the phenotypes of stress and addiction. As noted by Kreek (2008), one of the limiting factors in advancing genetics of addiction has been the lack of well-focused and detailed phenotyping of complex disorders (Kreek, 2008). Selective

phenotyping for stress- and cue-induced craving, such as the one performed in this sample, may be useful in elucidating genetic markers underlying risk for alcoholism initiation and maintenance through stress-based mechanisms. Further studies comparing subclinical and clinical samples are needed to more fully ascertain the clinical nature and implications of this behavioral finding.

Genetically, the present findings demonstrate an association between variation in a polymorphism of the CRH-BP gene, rs10055255, and stress-induced craving. This finding is consistent with the noted association between this polymorphism and enhanced negative stress reactivity at the mood level of analysis, such that homozygotes for the T allele at this locus reported higher stress-induced craving, stress-induced negative mood, and stress-induced tension, than A-allele carriers. As shown in Fig. 1, this tag SNP is in high LD with SNPs previously associated with resting EEG alpha power and alcohol use disorders (rs7728378 and rs1875999) in Caucasian samples (Enoch et al., 2008). A recent pharmacogenetic study has found rs10055255 to be associated with clinical response to citalopram (Binder et al., 2010). CRH-BP codes for a high-affinity binding protein for CRH, which in turn plays a central role in behavioral and physiological stress reactivity. These results extend previous findings implicating CRH-related peptides and addiction by testing a mechanistic link, namely stress reactivity and stress-induced craving, between the CRH-BP candidate gene and alcohol use outcomes.

Preclinical studies have convincingly demonstrated that CRF antagonists produce antistress effects (Heinrichs et al., 1994; Spina et al., 2000) and may play an important role in pharmacotherapy development for alcoholism (Heilig and Koob, 2007). A recent study demonstrated that selective blockade of CRF_2 receptor in the ventral tegmental area (VTA) attenuates stress-induced reinstatement of cocaine seeking and that CRH-BP located in the VTA mediates these effects (Wang et al., 2007). Likewise, blockade of CRH receptors produces stress-specific reductions in relapse risk (Heilig and Koob, 2007). Laboratory models such as the one employed in this study may be useful in identifying the biobehavioral mechanisms and determining initial efficacy of novel pharmacotherapies for addiction targeting the CRH system. These findings may also have pharmacogenetic implications for novel CRH-targeted pharmacotherapies. Importantly, theoretical models and preclinical findings have suggested that the involvement of the CRF system in stress sensitivity and negative emotionality is most salient during the later, more compulsive, stages of addiction (Koob, 2010). Hence, future studies of more severe clinical samples are clearly warranted as the present sample more closely approximates the early, impulsive, stage of alcohol use.

Regarding the Asn40Asp SNP of the OPRM1 gene, results revealed no genotype effects on stress reactivity or stress-induced craving. However, carriers of the Asp40 allele reported greater cue-induced alcohol craving during the neutral condition, when compared to the stressful imagery condition. This finding is in turn consistent with a previous cue-reactivity finding for this SNP (van den Wildenberg et al., 2007) and a more recent approach avoidance task in which carriers of the Asp40 allele showed greater approach bias toward alcohol, and more broadly, stronger approach bias toward positive stimuli (Wiers et al., 2009). This finding is also in line with some of our own work demonstrating a role of this SNP in subjective responses to alcohol in the laboratory (Ray and Hutchison, 2004) and in the natural environment (Ray et al., 2010b). In addition, a recent study has demonstrated that male Asp40 carriers displayed greater striatal dopamine release upon intravenous alcohol exposure, when compared to Asn40 homozygotes (Ramchandani et al., 2010). To the degree to which alcohol cues elicit striatal dopamine release, greater cue-reactivity among Asp40 carriers may be dopamine mediated. As the literature on the significance of this polymorphism to alcoholism etiology and treatment evolves, particularly the pharmacogenetics of naltrexone (Oroszi et al., 2008; Oslin et al., 2003; Ray and Hutchison, 2007), it appears as though mechanisms of reward, not punishment or stress, may be most salient to the risk conferred by this functional missense mutation.

These results should be considered in light of the study's strengths and limitations. The study is limited to a nonclinical sample of heavy drinkers and as such, replication in clinical samples is warranted. Moreover, the limited statistical power precluding type I error correction represents a limitation of the present study. The genotype selection for the CRH-BP gene was driven by a tag SNP approach, and future molecular studies should elucidate functional variants in this gene. This study focused primarily on experimental and self-report data. Additional studies of biological markers, such as cortisol and psychophysiological parameters, would extend the present findings. Nevertheless, the experience of stress and alcohol craving is a rather subjective one (Monti et al., 2004), such that the present study remains highly informative by capturing subjective parameters consistently associated with the addiction phenotypes of interest (Sinha, 2001, 2009). Study strengths include the controlled laboratory design utilizing a unique combination of 2 externally valid paradigms of stress and craving for alcohol. In addition, this study was theory driven in the selection of the ORPM1 and CRH-BP as candidate genes and seeks to translate preclinical findings and a theoretical addictions framework to studying a sample of risky drinkers.

On balance, these results suggest a novel contribution of CRH-BP to stress-induced craving and stress reactivity in the laboratory, which extends recent preclinical (Wang et al., 2007) and clinical (Enoch et al., 2008) findings suggesting a role for CRH-BP in addictions. These results also support the growing literature on the role of the Asn40Asp SNP of the OPRM1 gene and alcoholism phenotypes. Future studies translating these findings to clinical samples as well as to pharmacotherapy development efforts targeting the regulation of stress and craving mechanisms in alcoholism seem warranted.

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