ALCOHOLISM CLINICAL & EXPERIMENTAL RESEARCH

Subjective Response to Alcohol Among Alcohol-Dependent Individuals: Effects of the Mu-Opioid Receptor (*OPRM1*) Gene and Alcoholism Severity

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Background: Subjective response to alcohol has been examined as a marker of alcoholism risk. The A118G single-nucleotide polymorphism (SNP) of the mu-opioid receptor (*OPRM1*) gene has been previously associated with subjective response to alcohol in heavy drinkers. This study seeks to extend the literature by examining the effect of OPRM1 genotype on responses to alcohol in a sample of alcohol-dependent individuals. A secondary aim of this study is to examine alcoholism severity as a predictor of subjective responses to alcohol.

Methods: Nontreatment seeking problem drinkers (n = 295) were assessed in the laboratory for clinical dimensions of alcohol dependence. Following prospective genotyping, 43 alcohol-dependent individuals across the 2 genotype conditions (AA, n = 23 and AG/GG, n = 20) were randomized to 2 intravenous infusion sessions: 1 of alcohol (target breath alcohol concentration = 0.06 g/dl) and 1 of saline. Measures of subjective responses to alcohol were administered in both infusion sessions.

Results: Alcohol-dependent G-allele carriers reported greater alcohol-induced stimulation, vigor, and positive mood, as compared to A-allele homozygotes. There was no genotype effect on alcohol-induced sedation or craving. There was a statistical trend-level severity × alcohol interaction such that individuals at higher levels of severity reported greater alcohol-induced tension reduction.

Conclusions: These results support the hypothesis that OPRM1 genotype moderates the hedonic effects of alcohol, but not the sedative and unpleasant effects of alcohol, in a sample of alcohol-dependent patients. Results are discussed in light of a clinical neuroscience framework to alcoholism.

Key Words: Alcohol Dependence, Responses to Alcohol, Genetics, OPRM1, A118G SNP, Endophenotype.

TWIN AND ADOPTION studies have shown that the heritability of alcohol dependence (AD) may be as high as 50 to 60% (Kendler et al., 1997; Prescott and Kendler, 1999). However, the genetic architecture of AD is complex and remains largely elusive. In recent years, risk gene identification has progressed through the use of intermediate phenotypes for alcohol use disorders (Ducci and Goldman, 2008; Hines et al., 2005), including the subjective effects of alcohol (Ray et al., 2010b). To that end, the endogenous opioid system has been implicated in the pathophysiology of alcoholism as it modulates the reinforcing effects of alcohol via activation of mu-opioid receptors in the ventral tegmental area and nucleus accumbens, which in turn enhances extracellular concentrations of dopamine in the mesolimbic pathway (Gianoulakis, 2009; Koob and Kreek, 2007).

In light of the implication of endogenous opioids in alcohol-induced reward, several genetic association studies have focused on genetic variation in the mu-opioid receptor (*OPRM1*) gene as a plausible candidate locus for alcoholism phenotypes. In particular, a single-nucleotide polymorphism (SNP) of the *OPRM1* gene, the A118G SNP (rs17799971), has received significant attention given molecular evidence that this locus is a site of glycosylation. This nonsynonymous mutation results in an amino acid change from asparagine to aspartic acid, which in turn is thought to increase binding affinity for β-endorphin (Bond et al., 1998). An additional study has shown that this polymorphism affects gene expression (Zhang et al., 2005), yet the exact molecular mechanisms remain elusive. Genetic association studies have examined this polymorphism in relation to diagnostic phenotypes of alcohol and drug dependence with mixed results (Arias et al., 2006). However, an experimental study focusing on behavioral mechanisms of alcohol reward in a

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sample of heavy drinkers has shown that compared to A-allele homozygotes, G-allele carriers report greater subjective reinforcement from alcohol in the laboratory (Ray and Hutchison, 2004). Similar results were obtained in a naturalistic study of the effects of alcohol (Ray et al., 2010c). A study of nonhuman primates has demonstrated that male macaques carrying the SNP that is homologous to the Asp40 allele displayed increased alcohol-induced stimulation, consumed more ethanol (EtOH), and exhibited increased EtOH preference (Barr et al., 2007).

Further evidence for the relevance of the OPRM1 A118G polymorphism comes from cognitive neuroscience studies. A functional neuroimaging study revealed that G-allele carriers had greater hemodynamic response in mesocorticolimbic areas both before and after a priming dose of alcohol (Filbey et al., 2008). A recent study combining alcohol administration with positron emission tomography (Ramchandani et al., 2011) showed that G-allele carriers displayed a more potent striatal dopamine response to alcohol, compared to A-allele homozygotes. In conjunction, these studies support the biological plausibility of this polymorphism as a determinant of alcohol-induced reward, in terms of both hemodynamic response (Filbey et al., 2008) and dopamine release in the striatum following alcohol exposure (Ramchandani et al., 2011). This is consistent with the putative role of endogenous opioids in mediating the reinforcing effects of alcohol and suggests that ideal phenotypes to test candidate gene should probe the effects of alcohol on the reward circuitry (Ray et al., 2012).

The emerging research on the *OPRM1* gene in alcoholism has been fueled by its translational potential, particularly naltrexone pharmacogenetics (Heilig et al., 2010, 2011). Some clinical studies have shown that G-allele carriers may respond better to naltrexone (Anton et al., 2008; Oslin et al., 2003), while other studies have failed to replicate these findings (Gelernter et al., 2007). Laboratory studies have shown that G-allele carriers may experience greater blunting of alcohol reward on naltrexone (Ray and Hutchison, 2007; Setiawan et al., 2011), which in turn may explain its differential clinical efficacy in some trials. These pharmacogenetic effects have also been shown in nonhuman primates (Barr et al., 2011). While the clinical utility and mechanisms underlying the OPRM1 × Naltrexone interaction is not fully established (Ray et al., 2012), it is critical to extend the human laboratory findings from heavy/social drinking samples to individuals with AD.

The Present Study

This study extends the literature by testing the effects of the A118G SNP of the *OPRM1* gene on subjective response to alcohol in a sample of nontreatment seeking alcohol-dependent individuals. Participants were prospectively genotyped for the *OPRM1* gene and clinically ascertained for current AD status. Participants completed 2 randomized infusion sessions: one in which they received alcohol (target breath alcohol concentration (BrAC) = 0.06 g/dl) and one in which they received a saline control. It was hypothesized that G-allele carriers would display greater sensitivity to the stimulant and reinforcing effects of alcohol, as compared to saline, consistent with the role for the polymorphism in alcohol-induced reward. A secondary aim of this study was to examine the relationship between alcoholism severity and subjective response to alcohol. Given that the vast majority of alcohol administration studies to date have been conducted on heavy drinkers or at-risk samples, it was clinically relevant to ascertain how alcoholism severity is related to the subjective experience of alcohol. Consistent with the literature on the neurobiology of AD (Koob, 2003; Koob and Le Moal, 2008; Robinson and Berridge, 2001), it was hypothesized that individuals at earlier stages of alcoholism would report stronger positive and stimulant effects.

MATERIALS AND METHODS

Participants

Nontreatment seeking problem drinkers (n = 295) were recruited from the Los Angeles community through print and online advertisements. Inclusion criteria were (i) age between 21 and 65 years; (ii) self-identification of problems with alcohol; (iii) consuming a minimum of 48 standard drinks per month. Exclusion criteria were (i) in treatment for alcohol problems or seeking treatment, (ii) $\geq$21 days since last drink, (iii) history of bipolar disorder or any psychotic disorder, and (iv) CIWA-R score $\geq$10. The average age of the screening sample was 31.05 (SD = 10.49; range, 21 to 63), and the majority were male (73.5%). The ethnic background of the sample was White (55.6%), African American (23.5%), Asian (5.8%), Latino (13.7%), and Native American (1.4%). The majority of the screening sample met DSM-IV criteria for AD (n = 213, 72.2%), and all individuals enrolled in the alcohol administration met criteria for current AD.

Screening and Experimental Procedures

After initial telephone interview, eligible participants were invited to the laboratory for a screening session. After written informed consent, participants provided a saliva sample for DNA analyses and completed individual differences measures. Prospective genotyping was used to oversample for the A118G SNP of the *OPRM1* gene in the experimental portion of the study (Ray and Hutchison, 2004, 2007). Prior to the infusion sessions, participants attended a physical examination. From the total 295 participants screened in

Table 1. Alcohol Dependence Severity Measures, Factor Loadings

Scale	Factor loadings
DSM-IV alcohol abuse and dependence symptoms	**0.75**
ADS score	**0.83**
DrInC-2R score	**0.85**
PACS score	**0.74**
CIWA-Ar score	**0.48**

Factor loadings $\geq$0.40 are in boldface.

DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; ADS, Alcohol Dependence Scale; DrInC-2R, Drinkers Inventory of Consequences; PACS, Penn Alcohol Craving Scale; CIWA-Ar, Clinical Institute Withdrawal Assessment for Alcohol.

the laboratory, 48 were enrolled based on OPRM1 genotype and AD status and completed the physical exam. Of those, 45 were medically eligible, and 43 individuals were randomized. See Table 1 for demographics on the experimental sample ($n = 43$). The attrition from the screening sample of 295 to the randomized sample of 43 was the result of prospective genotyping for the G-allele of the *OPRM1* gene as well as the requirement that participants meet current criteria for AD.

Participants completed 2 randomized infusion sessions: 1 alcohol infusion and 1 saline control infusion. Alcohol administration was conducted using a single-blinded, randomized, counterbalanced, crossover design. Infusion sessions were separated by 1 to 2 weeks, with the observed time between infusions being 10.6 days. Participants were invited for an individual session of motivational interviewing upon completion of the study (34/43 completed the MI).

Measures

Individual Difference Measures. Alcohol use was assessed using the 30-day timeline follow-back (TLFB) interview (Sobell and Sobell, 1980). AD and the exclusionary psychiatric diagnoses were assessed using the Structured Clinical Interview for DSM-IV (SCID; First et al., 1995); the Clinical Institute Withdrawal Assessment for Alcohol (CIWA-Ar) assessed the presence and severity of withdrawal symptoms (Sullivan et al., 1989). Participants completed the Alcohol Dependence Scale (ADS) (Skinner and Allen, 1982), the Drinkers Inventory of Consequences (DrInC-2R) questionnaire (Miller et al., 1995), and the Penn Alcohol Craving Scale (PACS) (Flannery et al., 1999).

Alcohol Administration Procedures

All participants were required to have a BrAC of zero immediately prior to the alcohol administration, and participants who were regular smokers were allowed to smoke immediately prior to the infusions. Given the importance of effectively controlling blood alcohol levels (Li et al., 2001; O'Connor et al., 1998; Ramchandani et al., 1999), alcohol was administered intravenously using an established nomogram that takes into account participants' sex and weight (Ray and Hutchison, 2004; Ray et al., 2007). The infusion was performed using a 5% EtOH IV solution. Infusion rates were 0.166-ml/min × weight, in kilograms, for males, and 0.126-ml/min × weight, for females. Target BrACs were 0.02, 0.04, and 0.06 g/dl. These BrAC targets were selected to stay consistent with our previous work with heavy drinkers (Ray and Hutchison, 2004). Upon reaching each of the target BrAC levels, participants' infusion rates were reduced to half to maintain stable BrAC during testing. Specifically, participants were maintained at each target BrAC for an average of 7.32 minutes (SD = 3.03), during which they completed the study assessments. Participants were required to have a BrAC ≤ 0.02 g/dl before leaving the laboratory (or a BrAC = 0.00 g/dl if driving).

Alcohol Administration Measures. (i) The Biphasic Alcohol Effects Scale (BAES) captures feelings of alcohol-induced stimulation and sedation (Erblich and Earleywine, 1995; Martin et al., 1993); (ii) the Vigor and Positive Mood subscales of the Profile of Mood States (POMS) (McNair et al., 1971) were investigated given their previous association with OPRM1 effects in heavy drinkers (Ray and Hutchison, 2004; Ray et al., 2010c); and (iii) the Alcohol Urge Questionnaire (AUQ) is composed of 8 items assessing urge to drink (Bohn et al., 1995; MacKillop, 2006).

Genotyping

Saliva samples were collected under researcher observation for DNA analyses using Oragene saliva collection kits (DNA Genotek, Kanata, Ontario, Canada). 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 (Applied Biosystems, Foster City, CA). SNP sequencing was run on an AB 7900HT fast real-time PCR System and analyzed using the sequence detection systems (SDS) software version 2.3 (Applied Biosystems). Each run included 2 positive control samples (individual 2 in CEPH family 1347; Coriell Institute, Camden, NJ). Genotypes were automatically scored by the allele calling software and 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. In the screening sample ($n = 295$), the following OPRM1 genotypes were observed: AA, $n = 224$, AG, $n = 59$, and GG, $n = 10$ (2 samples could not be genotyped). In the experimental sample ($n = 43$), prospectively genotyped for the OPRM1 A118G SNP, genotypes were AA, $n = 23$, AG, $n = 18$, and GG, $n = 2$. Comparisons were made between A-allele homozygotes ($n = 23$) and G-allele carries, combining AG and GG ($n = 20$).

Data Analytic Plan

Analyses were performed using a multilevel regression-based framework (Singer, 1998) using PROC MIXED in SAS version 9.1 (SAS Institute Inc., Cary, NC) to test genotype group differences on subjective responses to alcohol. The critical p-value was set at $p < 0.05$ for all analyses. All analyses were modeled with individual intercepts and linear slopes across rising BrAC levels. Specifically, in the multilevel models, Alcohol and Genotype were Level 1 variables (nested within subjects), while subject and BrAC were Level 2 variables. The primary analyses of genotype effects examined the effects of *Alcohol*, a 2-level within-subjects factor (Alcohol vs. Saline, coded 0 and 1), *Genotype*, a 2-level between-subjects factor (A-allele hyomozygotes vs. G-allele carriers, coded 0 and 1), *Time,* a 4-level within-subjects factor (0 at baseline, 1 at BrAC = 0.02 g/dl or 18 minutes, 2 at BrAC = 0.04 g/dl or 43 minutes, and 3 at BrAC = 0.06 g/dl or 75 minutes), and their *interactions*. The dependent variables were measures of subjective response to alcohol (BAES and POMS) and alcohol craving (AUQ). Secondary analyses examined the effects of *Severity Factor*, *Alcohol*, and their *interaction* on measures of subjective response and craving. OPRM1 was retained in the alcohol severity models for outcome variables (i.e., subjective responses) in which genotype was a significant predictor. Alcoholism severity is tested in separate models given that the alcohol severity hypothesis is fairly novel and to our knowledge, similar models have not been reported in the literature to date. For those reasons, analyses of the alcoholism severity factor represent the secondary/exploratory aim of the study and were tested in separate models.

Alcoholism Severity Factor

To appropriately model the shared variance between the alcohol dependence severity indices (ADS, PACS, Symptom Count, DrInC-2R, and CIWA-Ar) and to minimize the number of statistical tests, a principal components analysis was conducted on the full sample of problem drinkers ($n = 295$) to derive factor scores capturing alcohol problem severity across a range of domains. The principal factor method followed by promax (oblique) rotation revealed 1 meaningful factor (Eigenvalue = 2.749) with each index loading onto the factor at 0.40 or greater and accounting for 55% of the total variance. The second factor fell below the 1.0 cutoff; thus, only the first factor was retained and interpreted to represent severity of AD. Participants' scores on this factor were used in analyses of the secondary aim regarding alcoholism severity and responses to alcohol. Factor loadings are presented in Table 1.

Table 2. Sample Demographics by OPRM1 Genotype

Variable[a]	AA (n = 23)	AG/GG (n = 20)	Test for difference
Gender (% female)	26.1	25.0	χ^2 (1) = 0.01, p = 0.94
Race (% Caucasian)	69.6	70.0	χ^2 (1) = 0.001, p = 0.98
Smoking status			χ^2 (2) = 6.01, p < 0.05
% Regular smoker	43.5	10.0	
% Occasional smoker	26.1	45.0	
% Nonsmoker	30.4	45.0	
Age	31.6 (10.8)	26.8 (7.1)	t (41) = 1.72, p = 0.09
Education	14.9 (4.3)	14.4 (1.8)	t (41) = 0.48, p = 0.64
DSM-IV alcohol dependence symptoms	5.0 (1.5)	4.4 (1.5)	t (41) = 1.32, p = 0.20
ADS score	42.2 (5.7)	42.6 (5.3)	t (41) = −0.25, p = 0.80
DrInC-2R score	52.7 (28.0)	49.0 (20.5)	t (41) = 0.49, p = 0.63
PACS score	20.0 (7.1)	19.9 (5.2)	t (41) = 0.03, p = 0.98
CIWA-Ar score	6.2 (4.7)	5.0 (4.1)	t (41) = 0.87, p = 0.39
Severity factor score	0.49 (0.9)	0.31 (0.8)	t (41) = 0.69, p = 0.50
Drinking days (past 30 days)	21.3 (7.9)	16.9 (6.5)	t (41) = 1.96, p = 0.06
Drinks per drinking day (past 30 days)	7.5 (3.2)	6.6 (2.5)	t (41) = 1.05, p = 0.30
Total drinks (past 30 days)	160.0 (94.6)	106.2 (45.6)	t (41) = 2.42, p < 0.05

[a]Standard deviations appear in parentheses.

DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; ADS, Alcohol Dependence Scale; DrInC-2R, Drinkers Inventory of Consequences; PACS, Penn Alcohol Craving Scale; CIWA-Ar, Clinical Institute Withdrawal Assessment for Alcohol.

RESULTS

Pretest Comparisons

As shown in Table 2, the 2 genotype groups did not differ on demographics or on measures of alcohol use and problems. There was a trend toward G-allele carriers being younger (p = 0.09) and reporting fewer drinking episodes in the past month (p = 0.06). In addition, A-allele homozygotes were more likely to be regular smokers (p < 0.05) and reported a higher total number of drinks over the past 30 days (p < 0.05), as compared to G-allele carriers. Based on the TLFB data collected immediately prior to each infusion session, the average time between infusion and last drink was 2.03 days (SD = 0.94). This interval did not differ by genotype group, t(41) = −0.26, p = 0.80. Likewise, the prevalence of the alcohol withdrawal symptom did not differ by genotype, χ^2(1) = 0.72, p = 0.40, suggesting that alcohol withdrawal is an unlikely confound in these analyses.

As a manipulation check, a series of simple models tested the effects of *Alcohol*, *Time*, and the *Alcohol* × *Time* interaction on the dependent variables of interest. As expected, results revealed a significant *Alcohol* × *Time* interaction on craving (AUQ, β = 0.21, SE = 0.11, t = 2.04, p < 0.05) and Positive Mood (POMS, β = 0.15, SE = 0.06, t = 2.70, p < 0.01). There were also trend-level *Alcohol* × *Time* interaction effects on stimulation (BAES, β = 1.94, SE = 1.01, t = 1.87, p = 0.06) and Vigor (POMS, β = 0.09, SE = 0.05, t = 1.62, p = 0.10). There was a main effect of alcohol on Sedation (BAES, β = 5.10, SE = 1.72, t = 2.95, p < 0.01). Effects were in the hypothesized direction, in that alcohol predicted stronger subjective responses than saline across time. There was no significant effect of genotype or Genotype × Alcohol interaction at baseline on any of the dependent variables of interest (ps > 0.10). Likewise, there was no significant effect of alcohol (vs. placebo) on baseline ratings of the BAES, POMS, and AUQ (ps > 0.10). In addition to the linear term, we also tested nonlinear effects (quadratic parameters) for the progression of subjective responses across rising BrACs. Doing so resulted in quadratic parameters that were not significant (main effect or interactions with genotype or alcohol) and were therefore excluded from subsequent analyses, while the linear parameters were retained.

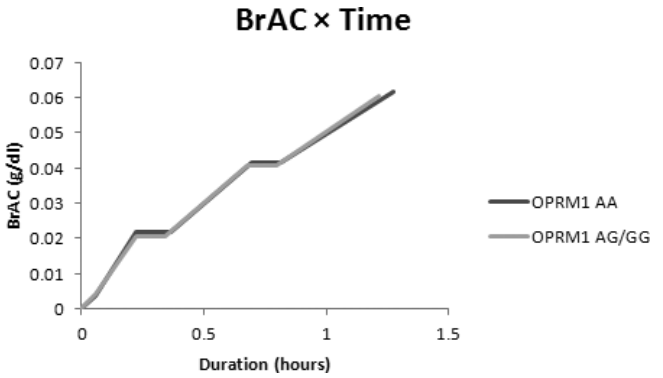

Fig. 1. Observed breath alcohol concentration (breath alcohol concentration [BrAC]) as a function of time (in hours) for both OPRM1 genotype groups.

Analyses then tested the effects of gender, ethnicity (white vs. nonwhite), and smoking status on subjective responses to alcohol (BAES and POMS) and alcohol craving (AUQ). Results revealed no effect of ethnicity on any of the dependent variables. There was a significant Alcohol × Smoking status effect on stimulation (β = −3.07, SE = 1.36, t = −2.26, p < 0.05) such that regular smokers reported less stimulation in the alcohol versus placebo condition. As for gender, there was a significant effect on stimulation (β = 6.09, SE = 2.46, t = 2.47, p < 0.05), Vigor (β = 0.29, SE = 0.13, t = 2.17, p < 0.05), and Positive Mood (β = 0.35, SE = 0.14, t = 2.58, p < 0.05) such that female participants reported greater alcohol-induced stimulation, vigor, and positive mood relative to males. Last, analyses of observed BrAC at each target level revealed no effect of gender (p = 0.18), ethnicity (p = 0.95), genotype (p = 0.09), or smoking status (p = 0.84) on BrAC during the active alcohol infusion. See Fig. 1 for observed BrAC × Time for both OPRM1 genotype groups.

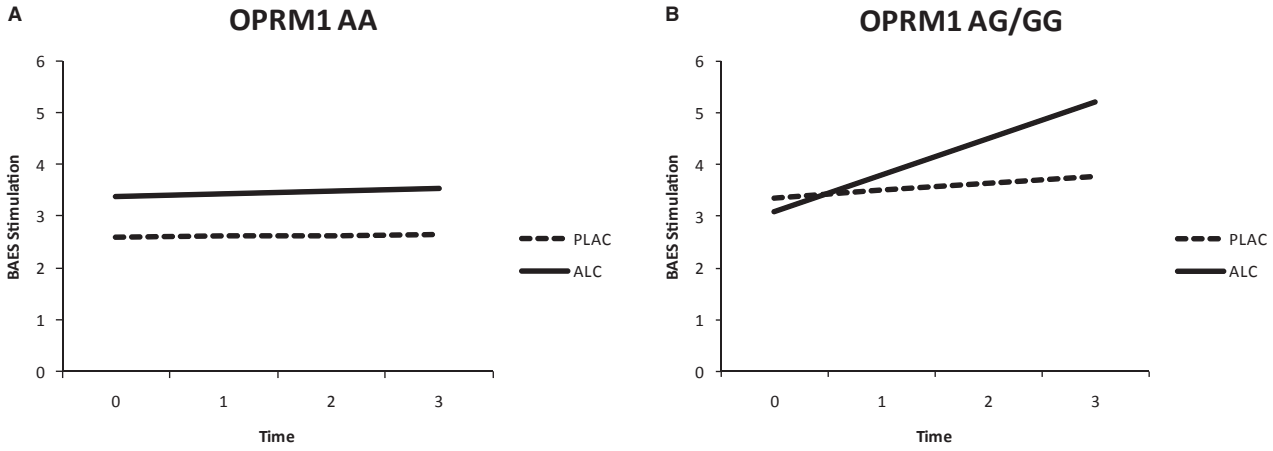

Fig. 2. Predicted values for stimulation as a function of time (breath alcohol concentration or assessment time) for alcohol (ALC) and placebo (PLAC) conditions, for A-allele homozygotes (**A**) and G-allele carriers (**B**).

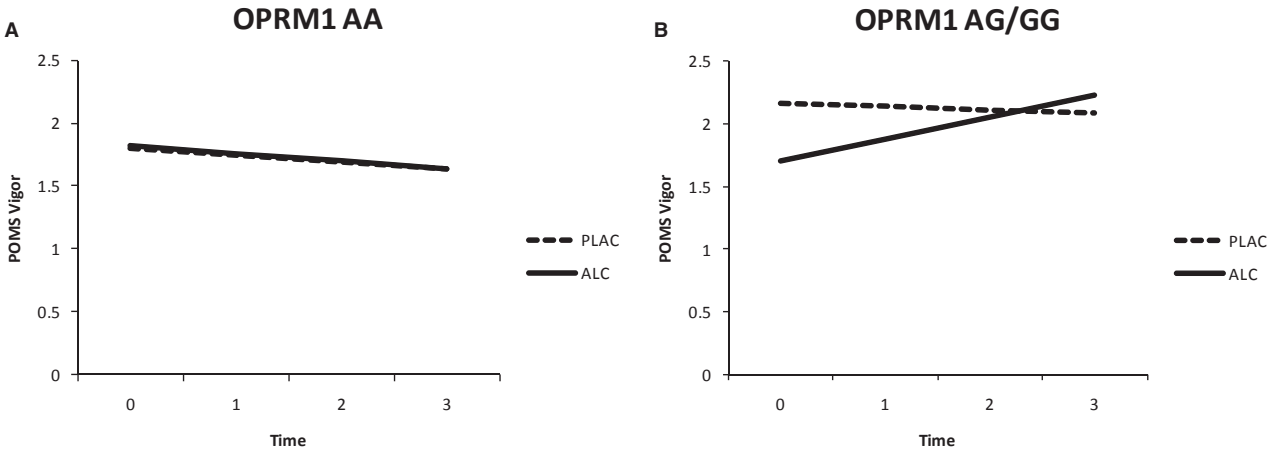

Fig. 3. Predicted values for vigor as a function of time (breath alcohol concentration or assessment time) for alcohol (ALC) and placebo (PLAC) conditions, for A-allele homozygotes (**A**) and G-allele carriers (**B**).

OPRM1 Effects

Analyses of the alcohol-induced *Stimulation* revealed a main effect of OPRM1 genotype ($\beta = 1.84$, SE = 0.93, $t = 1.97$, $p = 0.05$), an OPRM1 × Alcohol interaction ($\beta = -1.06$, SE = 0.52, $t = -2.04$, $p < 0.05$), and an OPRM1 × Alcohol × Time interaction ($\beta = 0.53$, SE = 0.28, $t = 1.92$, $p = 0.056$). As shown in Fig. 2, these results suggest that G-allele carriers report greater stimulation overall, greater stimulation in response to alcohol versus placebo, and greater increases in stimulation in the alcohol condition across time, as compared to A-allele homozygotes. A similar pattern of results was found for OPRM1 effects on alcohol-induced *Vigor* and *Positive Mood*. Analyses of alcohol-induced *Vigor* revealed a main effect of OPRM1 genotype effect ($\beta = 0.85$, SE = 0.37, $t = 2.29$, $p < 0.05$), an OPRM1 × Alcohol interaction ($\beta = -0.48$, SE = 0.19, $t = -2.49$, $p < 0.05$), and an OPRM1 × Alcohol × Time interaction ($\beta = 0.21$, SE = 0.10, $t = 2.00$, $p < 0.05$) (see Fig. 3). Likewise, for *Positive Mood,* there was a main effect of OPRM1 genotype effect ($\beta = 0.60$, SE = 0.26, $t = 2.31$, $p < 0.05$) and an OPRM1 × Alcohol interaction ($\beta = -0.33$, SE = 0.12, $t = -2.49$, $p < 0.01$).There was no OPRM1 genotype effect on alcohol-induced *Sedation* (OPRM1 × Alcohol: $\beta = -1.74$, SE = 2.02, $t = -0.86$, $p = 0.39$). Together, these results provide support for the initial hypothesis that OPRM1 genotype moderates the hedonic, but not the sedative, effects of alcohol.

To probe for the OPRM1 effects reported above, all analyses were repeated incorporating alcoholism Severity as a covariate. All the findings reported above remained statistically significant when Severity was added to the models. To control for population stratification effects, all models were repeated in Caucasians only ($n = 30$) and the results supported the original findings both in magnitude and direction. For stimulation, there was an OPRM1 × Alcohol interaction ($\beta = -9.46$, SE = 4.37, $t = -2.17$, $p < 0.05$). For Vigor, there were an OPRM1

main effect ($\beta = 0.85$, SE = 0.35, $t = 2.45$, $p < 0.05$) and an OPRM1 × Alcohol interaction ($\beta = -0.34$, SE = 0.15, $t = -2.29$, $p < 0.05$). And for Positive Mood, there were an OPRM1 main effect ($\beta = 0.98$, SE = 0.33, $t = 3.03$, $p < 0.01$) and an OPRM1 × Alcohol interaction ($\beta = -0.49$, SE = 0.15, $t = -3.26$, $p < 0.01$). Further, given the genotype group differences on smoking status, all analyses were repeated while controlling for current smoking status. Doing so resulted in all OPRM1 effects remaining statistically significant. These analyses also suggested significant effect of smoking status on self-reports of stimulation ($\beta = -4.64$, SE = 1.99, $t = -2.34$, $p < 0.05$) and Vigor ($\beta = -0.28$, SE = 0.14, $t = -2.05$, $p < 0.05$), such that regular smokers reported less stimulation and vigor across alcohol and saline infusions. Last, controlling for sex, sex × alcohol, days since last drink, number of drinking days in the past 30 days, and total number of drinks in the past 30 days, and the DSM-IV symptom of tolerance did not alter any of the significant OPRM1 effects.

Alcoholism Severity and Subjective Response to Alcohol

To test the secondary aim, a series of models tested the effects of *Severity* (i.e., *factor score*), *Alcohol*, *Time*, and the *Alcohol* × *Severity* interaction on the dependent variables of interest. Results revealed a main effect of Severity on Sedation ($\beta = 6.65$, SE = 2.40, $t = 2.77$, $p < 0.01$) and Craving ($\beta = 0.58$, SE = 0.29, $t = 2.01$, $p < 0.05$) across both infusions. However, there was no *Alcohol* × *Severity* interaction for either Sedation ($\beta = -1.02$, SE = 1.16, $t = -0.88$, $p = 0.38$) or Craving ($\beta = 0.02$, SE = 0.13, $t = 0.13$, $p = 0.90$). There were no main effects of Severity or *Alcohol* × *Severity* interactions on measures of the hedonic effects of alcohol, namely stimulation, vigor, and positive mood ($ps > 0.10$). Together, these results suggest that individuals at higher levels of alcoholism severity reported more sedation and alcohol craving during the infusions, yet these effects were not unique to the alcohol condition.

The tension subscale of the POMS was examined as a post hoc comparison for the effects of severity on the negative reinforcing properties of alcohol (i.e., tension reduction). Results revealed a trend-level *Alcohol* × *Severity* interaction ($\beta = 0.08$, SE = 0.04, $t = 1.92$, $p = 0.055$) such that individuals at higher levels of severity reported greater alcohol-induced tension reduction.

DISCUSSION

This study examined the moderating effects of the A118G SNP of the *OPRM1* gene on subjective responses to alcohol in a sample of alcohol-dependent patients. Consistent with the findings from heavy drinking samples (Ray and Hutchison, 2004; Ray et al., 2010c), clinically diagnosed alcohol-dependent G-allele carriers reported greater sensitivity to the stimulant and hedonic effects of alcohol. The use of an alcohol infusion to minimize pharmacokinetic variation and the addition of a placebo infusion are important as they allow for greater confidence in attributing these results to the pharmacological effects of alcohol. Moreover, the extension of these findings to a sample of individuals with alcoholism is important given that much of the mixed findings for the role of this polymorphism in alcoholism etiology, and treatment may be due to heterogeneity across samples and endpoints.

An important aspect of these findings is the specific nature of the observed genotype group differences. OPRM1 effects were selective for the positive and rewarding effects of alcohol, as compared to the sedative and unpleasant effects, as this polymorphism putatively mediates alcohol-induced dopamine release in the striatum (Ramchandani et al., 2011). In a previous alcohol administration study, a factor analysis of subjective responses to alcohol found that stimulation, vigor, and positive mood loaded into a common "Positive Reinforcing" factor, with separate "Negative Reinforcement," and "Punishment" factors (Ray et al., 2009). These results help further refine the literature by suggesting that the OPRM1 effects may be specific to alcohol reward and that these effects, both behavioral and genotypic, are present in alcohol-dependent samples.

In translating these findings in relation to AD, recent developments in the field have allowed for further refinement of the construct of subjective response to alcohol and worthy of consideration in this study and others. Specifically, we have previously argued that subjective response to alcohol can be either protective, when they are primarily aversive in nature, or a risk factor for further drinking and alcoholism, when the predominant subjective experience is one of reward and stimulation (Ray et al., 2009, 2010a,b). A recent meta-analysis of alcohol administration studies found support for 2 distinct mechanisms of risk, one marked by positive reinforcement and one marked by low level of response to alcohol, which is characterized by relative insensitivity to the aversive and punishing effects of alcohol (Quinn and Fromme, 2011). Moreover, a recent study combining alcohol administration with a longitudinal follow-up revealed that participants reporting more of the stimulant effects of alcohol and less of the sedative effects in the laboratory had a higher frequency of binge drinking, which was in turn a risk factor for the development of alcoholism at follow-up (King et al., 2011a). These recent findings have called for a paradigm shift in the interpretation of subjective response to alcohol and its etiological and clinical significance (King et al., 2011b). The present study builds upon this emerging literature to suggest that greater consilience in the OPRM1 findings may be reached by focusing on the genotype interactions with the stimulant and rewarding dimensions of alcohol's effects.

More broadly, this study also provides further support for an endophenotype approach in addiction genetics, which seeks to map the pathways from genetic risk to clinical risk by focusing on mechanistic intermediary processes that are more proximal to genetic variation (Gottesman and Gould, 2003; Ray et al., 2010b). Putative advantages of this

approach are greater reliability across studies, the presence of larger effect size relationships, and improved insights into the neurobiological and psychological mechanisms of risk. These are clearly evident in the current study, which extends previous human laboratory findings in a modestly sized sample and suggests that the role of the OPRM1 A118G polymorphism in relation to AD is by way of its modulation of alcohol-induced reward.

The secondary aim of this study was to examine alcoholism severity as a predictor of subjective responses to alcohol in the laboratory. To that end, results suggested a positive association between alcoholism severity and self-reported sedation and alcohol craving during the infusion, although the effects were seen across both alcohol and placebo conditions. In other words, these effects were not specific to alcohol. The original hypothesis based on the literature on alcoholism neurobiology (Koob, 2003; Koob and Le Moal, 2008; Robinson and Berridge, 2001) was that individuals at later stages of alcoholism would experience fewer positive, hedonic, and stimulant effects. While there was no evidence of a negative association between severity and the rewarding effects of alcohol, that may in part be due to the nature of the sample. Specifically, individuals with a CIWA-Ar score ≥ 10 were excluded from the experimental arm of the study, as it may have been unsafe for them to abstain prior to the infusion sessions. As such, more severe alcohol-dependent patients may have been excluded, thereby reducing sample variability and power to detect significant effects. It is also plausible that alcohol-specific effects were not detectable as a result of the modest main effect of alcohol (vs. saline). Interestingly, the post hoc test showing a trend toward greater tension reduction among more severely dependent patients is consistent with the neurobiological models predicting negative reinforcement as a primary motivational drive toward alcohol use at later stages of addiction (Koob, 2009; Koob and Le Moal, 2005). Likewise, albeit not alcohol-specific, higher levels of craving and sedation are also consistent with later stages of alcoholism. In summary, while the results of the secondary aim are far from conclusive, they represent a much needed step toward the translation of findings to clinical samples by hypothesis generation and testing that is informed by the neuroscience of addiction.

The present findings must be interpreted in light of the study's strengths and limitations. Strengths include the highly controlled alcohol administration procedures and the addition of a saline control. The clinically ascertained sample of alcohol-dependent patients and the prospective genotyping also strengthen the study's conclusions. Study limitations include the small sample size, which was sufficient for testing the primary hypotheses, but did not permit subgroup analyses by race or sex. Additionally, the dose of alcohol, while shown to have the expected effect on subjective responses, was relatively modest, and additional studies testing higher alcohol doses are warranted, particularly in alcohol-dependent samples for which the development of tolerance is an issue. Of note, the diagnostic symptom of tolerance was met by 84% of participants such that analyses of its effect of subjective responses to alcohol were largely underpowered. Another limitation is the fact that participants accurately guessed the alcohol and saline conditions, thereby raising the potential for expectancy effects. At least 1 study has found that G-allele carriers report greater expectancy for the rewarding effects of alcohol (i.e., drinking to enhance positive affect) (Miranda et al., 2010). Further, the absence of taste, visual, and olfactory alcohol cues in the alcohol infusion may have dampened the overall experience of alcohol craving, consistent with previous comparisons of oral versus intravenous alcohol administration (Ray et al., 2007). Last, the OPRM1 $\times$ Alcohol $\times$ Time interaction predicting stimulation was marginal ($p = 0.056$) and should be interpreted with caution and in the context of the totality of the findings.

In conclusion, the present study extends the literature on the effects of the *OPRM1* gene on subjective response to alcohol by demonstrating that alcohol-dependent G-allele carriers report greater alcohol-induced stimulation, vigor, and positive mood, consistent with the hypothesized role of this polymorphism on the rewarding effects of alcohol. This study also advances clinical neuroscience of alcoholism by testing the role of ADS on subjective responses to alcohol, in light of neurobiological theories of changes in motivational drives across alcoholism stages. Future studies are warranted to extend this translational approach in large samples, with a wider range of alcoholism severity, and at larger doses of alcohol.

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