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Polymorphisms of the μ-Opioid Receptor and Dopamine D_4 Receptor Genes and Subjective Responses to Alcohol in the Natural Environment

Lara A. Ray
Brown University and University of California, Los Angeles

Robert Miranda, Jr., and Jennifer W. Tidey
Brown University

John E. McGeary
Providence Veterans Affairs Medical Center, Providence, RI, and Brown University

James MacKillop
Brown University and University of Georgia

Chad J. Gwaltney
Brown University

Damaris J. Rohsenow, Robert M. Swift, and Peter M. Monti
Providence Veterans Affairs Medical Center, Providence, RI, and Brown University

Polymorphisms of the μ-opioid receptor (OPRM1) and dopamine D_4 receptor (DRD4) genes are associated with subjective responses to alcohol and urge to drink under laboratory conditions. This study examined these associations in the natural environment using ecological momentary assessment. Participants were non-treatment-seeking heavy drinkers (n = 112, 52% female, 61% alcohol dependent) who enrolled in a study of naltrexone effects on craving and drinking in the natural environment. Data were culled from 5 consecutive days of drinking reports prior to medication randomization. Analyses revealed that, after drinking, carriers of the Asp40 allele of the OPRM1 gene reported higher overall levels of vigor and lower levels negative mood, as compared to homozygotes for the Asn40 variant. Carriers of the long allele (i.e., ≥7 tandem repeats) of the DRD4 endorsed greater urge to drink than homozygotes for the short allele. Effects of OPRM1 and DRD4 variable-number-of-tandem-repeats genotypes appear to be alcohol dose-dependent. Specifically, carriers of the DRD4-L allele reported slight decreases in urge to drink at higher levels of estimated blood alcohol concentration (eBAC), and Asp40 carriers reported decreases in vigor and increases in negative mood as eBAC rose, as compared to carriers of the major allele for each gene. Self-reported vigor and urge to drink were positively associated with alcohol consumption within the same drinking episode. This study extends findings on subjective intoxication, urge to drink, and their genetic bases from controlled laboratory to naturalistic settings.

Keywords: OPRM1, DRD4, urge to drink, alcohol, EMA

Alcohol intoxication is a complex pharmacological process that involves multiple neurotransmitter systems and produces a host of physiological and behavioral effects. These effects, in turn, govern the reinforcing properties of drinking (Grobin, Matthews, Devaud, & Morrow, 1998; Herz, 1997). In light of prospective evidence linking individual differences in alcohol sensitivity to the development of alcohol use disorders (Schuckit & Smith, 1996, 2000), identifying genetic influences on acute subjective responses to alcohol is an emerging area of scientific inquiry. Alcohol ingestion triggers opioid activity, which inhibits GABA neurons and facil-

Lara A. Ray, Center for Alcohol and Addiction Studies, Brown University, and Department of Psychology, University of California, Los Angeles; Robert Miranda, Jr., Jennifer W. Tidey, and Chad J. Gwaltney, Center for Alcohol and Addiction Studies, Brown University; John E. McGeary, Damaris J. Rohsenow, Robert M. Swift, and Peter M. Monti, Providence Veterans Affairs Medical Center, Providence, RI, and Center for Alcohol and Addiction Studies, Brown University; James MacKillop, Center for Alcohol and Addiction Studies, Brown University, and Department of Psychology, University of Georgia.

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Correspondence concerning this article should be addressed to Lara A. Ray, University of California, Los Angeles, Department of Psychology, 1285 Franz Hall, Box 951563, Los Angeles, CA 90095-1563. E-mail: lararay@psych.ucla.edu

itates dopamine release (Erickson, 1996; Herz, 1997; Kreek, 1996). This cascade of neurochemical actions subserves the positively reinforcing effects of alcohol (Bond et al., 1998; Herz, 1997; Wise & Bozarth, 1987). Consequently, genes of putative relevance to these systems are prime research targets.

Of myriad genes involved in the opioid system, the single nucleotide polymorphism (SNP) A118G (*rs1799971*)[1] of OPRM1 is the most widely studied due, in part, to evidence suggesting that it exerts functional effects on the receptors (Bond et al. 1998; Zhang, Wang, Johnson, Papp, & Sadee, 2005). Although association findings between this SNP and alcohol dependence are mixed (for a meta-analysis, see Arias, Feinn, & Kranzler, 2006), controlled laboratory studies have indicated that individuals with the Asp40 allele of this gene report higher subjective feelings of intoxication, stimulation, sedation, and positive mood across rising levels of blood alcohol concentration (BAC), as compared to those with the Asn40 allele (Ray & Hutchison, 2004, 2007). In addition, male carriers of the Asp40 allele report higher levels of alcohol craving following alcohol cue exposure, as compared to those homozygous for the Asn40 allele (van den Wildenberg, Wiers, et al., 2007). Thus, this polymorphism may be associated with the reinforcing or stimulant effects of alcohol.

The dopamine D_4 receptor gene (DRD4) is expressed in brain regions associated with attention, cognition, and drug reward (Oak, Oldenof, & Van Tol, 2000; Wise & Bozarth, 1987). It contains a 48-base-pair (48-bp) variable number of tandem repeats (VNTR) in exon III, with three common length variants (i.e., two, four, and seven repeats; Grady et al., 2003; Van Tol et al., 1992). Importantly, the seven-repeat allele blunts intracellular response to dopamine (Asghari et al., 1995) and attenuates inhibition of intracellular cyclic AMP (Oak et al., 2000). In terms of alcohol-related phenotypes, the DRD4 VNTR has produced equivocal findings. Although the direct association between DRD4 and alcohol diagnosis has yielded largely negative results (Tyndale, 2003), carriers of a long (L) allele (i.e., $\geq$7 repeats) exhibited higher alcohol craving and consumption in the laboratory, as compared to homozygotes for the short (S) allele (i.e., $<$7 repeats; Hutchison et al., 2002; MacKillop, Menges, McGeary, & Lisman, 2007; McGeary et al., 2006). However, a recent study failed to replicate these findings (van den Wildenberg, Janssen, Hutchison, van Breukelen, & Wiers, 2007).

Inasmuch as laboratory studies of intermediate phenotypes, such as craving and sensitivity to alcohol, potentially afford a more sensitive test of gene–disorder associations than complex alcohol use disorder diagnoses (Gottesman & Gould, 2003; Hines, Ray, Hutchison, & Tabakoff, 2005), studies have suggested that the Asp40 allele of the OPRM1 gene and L allele of the DRD4 VNTR may be associated with greater sensitivity to the reinforcing effects of alcohol and craving, which in turn may influence their susceptibility to problematic alcohol use. Research supporting these hypotheses, however, has come exclusively from highly controlled laboratory settings. Therefore, it remains unknown whether these findings would generalize to the natural environment.

The purpose of the present study was to build upon laboratory research by examining genotype effects on subjective responses to alcohol and craving (i.e., urge to drink) in the natural environment using ecological momentary assessment (EMA) technology. This approach involves collecting data in real time about momentary events as they occur in the participants' natural environment by having them use handheld electronic diaries (EDs) to monitor target behaviors while engaged in their usual daily activities. Momentary assessments are particularly important when the phenomena of interest are subject to rapid change (Shiffman, Stone, & Hufford, 2008), such as urge to drink and the acute subjective effects of alcohol. We conceptualize EMA as a parallel and complementary assessment tool to more controlled laboratory methods. Each method has its own strengths and weaknesses. EMA emphasizes ecological validity, which may yield different findings than laboratory research because contexts are more complex and realistic. EMA also affords the ability to capture a host of environmental and contextual factors (e.g., setting, whether others were drinking) that can be examined and accounted for as time-varying covariates. We hypothesized that carriers of the Asp40 allele of OPRM1 and of the L allele of DRD4 would report greater urge to drink and more reinforcing subjective responses to alcohol than participants homozygous for the major allele of each gene.

Method

Participants

Participants were non-treatment-seeking heavy drinkers recruited from the community through newspaper advertisements for study of naltrexone's effects on drinking, urges, and mood in the natural environment (for details, see Tidey et al., 2008). This study focused on previously unreported data from the baseline period to avoid the complicating placebo and medication effects. Eligibility criteria included 21 years of age, drinking at least 4 days per week, and reporting heavy drinking on at least 2 days per week on average over the preceding month ($>$6 standard drinks for men, $>$4 standard drinks for women; Flannery et al., 2002). Exclusionary criteria included abuse of or dependence on drugs other than nicotine and alcohol, current interest in or past treatment for alcohol problems, positive urine screen for opiates or cocaine (positive screens for marijuana were enrolled), positive pregnancy test, nursing, not using birth control (women), and medications or medical conditions that counterindicated naltrexone treatment. A subset of participants provided consent for DNA collection and represents the current sample. As genotyping in this study began about 12 months after recruitment started, the sample sizes for the DRD4 and OPRM1 analyses are $n = 112$ and $n = 105$, respectively. See Table 1 for participant characteristics and genotype comparisons. To assess for potential selection bias due to the fact that only a subset of patients participated in the DNA collection, consenters ($n = 112$) and nonconsenters ($n = 64$; 38 who could not be contacted, 26 who refused consent) were compared on the baseline characteristics.

[1] Note that although this SNP is referred to in the literature, as well as in this article, as the Asn40Asp (or the A118G SNP), this designation has been recently updated in the public bioinformatics databases (ABI, NCBI, HapMap) as it has been determined that the OPRM1 protein may contain an additional 62 amino acids. The new designation of this SNP based on the National Center for Biotechnology Information Human Genome Assembly 36 is Asn102Asp (or A355G; see http://www/mcbi.nlm.nih.gov/SNP).

Table 1
Baseline Participant Characteristics by Genotype: Means or Percentages (With Standard Deviations in Parentheses)

	OPRM1		DRD4	
Variable	Asn40 (n = 72)	Asp40 (n = 33)	S (n = 69)	L (n = 43)
Age	28.0 (10.9)	30.5 (12.1)	28.2 (11.4)	31.2 (12.3)
Gender (% male)	43.1%	63.6%*	56.5%	34.9%*
Alcohol dependent	58.3%	66.7%	63.8%	58.1%
Caucasian	93.1%	93.9%	95.7%	88.4%
Years of education	14.8 (1.5)	15.0 (1.7)	15.0 (1.5)	14.6 (1.5)
Smoker	33.3%	36.4%	30.4%	44.2%
Drinks per day	4.3 (1.9)	4.8 (2.5)	4.5 (2.2)	4.4 (2.0)
Drinks per drinking day	6.8 (2.2)	7.2 (3.6)	7.1 (2.7)	6.5 (2.7)
Drinking days	63.0% (16.3%)	67.0% (17.2%)	62.7% (17.4%)	69.3%* (16.4%)
Heavy drinking days	45.0% (18.1%)	43.1% (19.2%)	43.3% (18.6%)	47.4% (18.2%)
DrInC-2R score	21.7 (17.4)	19.7 (14.7)	19.5 (15.2)	23.7 (17.9)

Note. Comparisons were conducted separately for DRD4 and OPRM1 genotypes. DrInC-2R = the Drinker Inventory of Consequences (Miller, Tonigan, & Longabaugh, 1995) in its recent drinking version. The DrInC-2R assesses the number and frequency of various drinking-related negative consequences. Range = 0–85. S = short allele; L = long allele.
* $p < .05$.

Procedures

Participants were told that the purpose of the 5-week assessment was to study the effects of a medication on urge to drink, mood, and alcohol use. They were not given instructions to reduce or otherwise alter their drinking. After providing informed consent, participants completed the individual-difference measures. Participants were then trained to use EMA on handheld computers and initiated their daily EMA recording, completing assessments multiple times per day. After 2 practice days, their EMA data were downloaded and reviewed with them for compliance. Participants were instructed to self-initiate assessments at the beginning and end of drinks when they occurred and to respond to all audible prompts immediately. The data collected over the next 5 days constituted the premedication baseline, which was the focus of the present study.

At the end of the 5-day baseline period, 1 participant failed to meet the minimal EMA compliance criterion of responding to at least 50% of random prompts and was discontinued from the study. The placebo lead-in portion of the study started at Week 2 and randomization to the medication portion at Week 3, as described elsewhere (Tidey et al., 2008). DNA collection was performed via buccal swabs using established procedures (Freeman et al., 1997; Lench, Stainer, & Williamson, 1988).

Assessments

Individual-difference measures included a demographics questionnaire and the 90-day timeline followback interview to assess quantity and frequency of drinking (Sobell & Sobell 1992). Alcohol diagnoses were based on the criteria of the Structured Clinical Interview for *DSM–IV* Axis I Disorders—Patient Version (First, Spitzer, & Gibbon, 1995). The Drinker Inventory of Consequences (DrInC-2R; Miller, Tonigan, & Longabaugh, 1995) assessed the occurrence of various drinking-related negative consequences.

EMA Assessments and Compliance

The ED system was implemented on handheld computers (PalmPilot IIIxe; Palm, Sunnyvale, CA) running software designed for this study (invivodata, Pittsburgh, PA). Participants completed assessments on the ED (a) upon awakening (morning report); (b) in response to audible prompts presented at random times during the waking day, approximately five times per day (random prompts); (c) at the start of each drink episode (begin drink report); and (d) when completing each of the first two drinks of each drinking episode (end drink report). Given that the present study seeks to elucidate genetic determinants of subjective response to alcohol, the analyses focused on assessments during the reported drinking episodes (below).

Begin and end drink reports. Data were collected before and after the first two drinks of a drinking episode due to concerns that higher levels of intoxication could decrease measurement reliability.[2] In the begin drink report, participants were asked to rate their urge and mood "just before drinking." The end drink report assessed mood and urge to drink at the current time (i.e., "right now") and included questions about the type and quantity of beverage consumed. The following are the dependent variables used in this study: (a) Mood items were derived from the Profile of Mood States (McNair, Lorr, & Droppleman, 1971) to capture the following mood dimensions: vigor (items: aroused, energetic) and negative mood (items: miserable, sad, contented—the last item was reverse scored). These items mirrored previous reports of alcohol's subjective effects (Ray & Hutchison, 2004, 2007), were rated on scales from 0 (*Not at all*) to 10 (*Extremely*), and were combined

[2] Please note that the decision to truncate reporting at two drinks was based on group consensus and the expectation that alcohol intoxication over two drinks would impair judgment and, hence, reduce the accuracy of EMA reporting. Due to those concerns, the investigative group chose a sampling strategy (Shiffman et al., 2008), in which we decided that responses to only the first two drinks would be assessed, in lieu of a coverage strategy, in which one would attempt to assess subjective responses to every drink of the day. Whether sampling more than two drinks significantly reduces the reliability of the EMA data remains an open empirical question, one that ought to be examined in future research in which reporting is not truncated to the first two alcoholic drinks.

into a mean score for each mood dimension. (b) Urge to drink was rated on 0 (*No urge*) to 10 (*Strongest ever*) scales. See Table 2 for average scores on each dependent variable at begin and at end drink 2 reports.

To reduce participant burden and increase compliance with the EMA protocol, assessments proceeded as follows: (a) Urge to drink was assessed at the begin drink report and again at the end drink report for the first and second drinks; (b) all mood items were assessed at the begin drink report and then at the end drink report for the second drink only. At the end drink report for the first drink, participants reported on contextual and environmental factors surrounding the drink episode, with questions such as (a) "Where were you?" (i.e., setting), with possible answers being "home," "work," "school," "other's home," "bar," "restaurant," "liquor store," "vehicle," "outside," or "other"; (b) "Were others drinking?", with possible answers being "yes," "no," or "others in view"; and (c) "How long before the drink was your last cigarette?", with possible answers ranging from 0 to 99 min. These episode-varying contextual and environmental factors were examined in the analyses as time-varying covariates. As described below, BAC was estimated for each drink and used in all analyses.

Estimated blood alcohol concentration. Given that previous studies have shown that the subjective effects of alcohol are alcohol dose-dependent (Anton, Drobes, Voronin, Durazo-Avizu, & Moak, 2004; Drobes, Anton, Thomas, & Vornin, 2004; McCaul, Wand, Stauffer, Lee, & Rohde, 2001; Ray & Hutchison, 2007), BAC was estimated in this study on the basis of alcoholic beverage type and amount, gender, weight, and time elapsed from alcohol consumption. Participants were asked if they drank beer, wine, wine cooler, fortified wine, mixed drink, or straight liquor and reported the number of ounces consumed (possible range = 0–40 ounces). On the basis of beverage type and quantity, those drinks were converted into standard drinks using the standard drink definition provided by National Institute on Alcohol Abuse and Alcoholism (2005), such that a standard drink (containing 14 g of alcohol) is defined as 12 ounces of beer, 5 ounces of wine, 12 ounces of wine cooler, 3.5 ounces of fortified wine, or 1.5 ounces of hard liquor (in a mixed drink or as straight liquor).

On average, the drinks consumed by participants in this study exceeded the definition of a standard drink (1.12, $SD = 0.36$, standard drinks for Drink 1 and 1.13, $SD = 0.35$, for Drink 2), which is consistent with prior research (Kaskutas & Graves, 2000). To estimate BAC, we used a nomogram that takes into account the number of standard drinks, the time from the end of drink consumption to the end drink report, gender, and weight to estimate BAC at the time of first and second drink reports within a single drinking episode. The average estimated BAC (eBAC) was 0.023 g/dl ($SD = 0.013$) at the end of Drink 1 and 0.042 g/dl ($SD = 0.027$) at the end of Drink 2. These results are consistent with previously published guidelines for calculating BAC by varying levels of gender, weight, drinks, and time (Brick, 2006; Fisher, Simpson, & Kapur, 1987).

EMA compliance criteria. Noncompliance with the monitoring protocol (e.g., not entering drinks in real time) may threaten the validity of the EMA data, and there was no way to ensure that all drink reports used in analysis were completed at the appropriate time. However, three methods were used to increase the probability that the drink reports included in this study were valid. First, we included a question in a morning report that asked the participant if he or she had forgotten to enter any drinks the previous day. On only 3.9% of days did participants endorse failing to enter drinks. Second, participants were asked in the begin and end drink reports to indicate how long after the beginning, or end, of the drink the assessment was initiated. Reports were discarded if the participant indicated that the start or end of the drink had occurred more than 10 min before the initiation of the report. Furthermore, we identified participants who were noncompliant with other aspects of the study. It might be expected that these participants would also be noncompliant with the drink reports. Poor compliance was operationally defined as (a) completing less than 50% of audibly prompted assessments per week; (b) using the ED sleep function, which allowed participants to turn the device off while sleeping, for 13 or more hr on 4 or more days in a week; and (c) suspending audible prompting for more than 14 hr in a week. If any of these criteria were met, the participant's data were not used in the analyses. In the entire sample, compliance with the protocol was very high (e.g., approximately 80% compliance with audible prompts; Tidey et al., 2008).

DNA Analyses

The Asn40 SNP in the OPRM1 gene was assayed using a modification of restriction fragment length polymorphism procedures reported by Bergen et al. (1997). Samples were genotyped again using the ABI Taqman assay for rs1799971 to ensure that the high frequencies of the Asp40 variant found were not due to genotyping error. The 48-bp VNTR in exon III of the DRD4 gene was assayed using modifications of previously reported methods (Sander et al., 1997). Consistent with the existing literature, participants were grouped by OPRM1 status such that the Asp40

Table 2
Scores on the Dependent Variables at Begin Drink Report and at End Drink 2 Report (M ± SD) by Genotype

	OPRM1				DRD4			
	Asn40		Asp40		Short		Long	
Variable	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Vigor	5.70 (2.14)	5.99 (2.11)	5.22 (2.07)	5.33 (2.25)	5.35 (2.11)	5.80 (2.25)	5.85 (2.13)	5.78 (2.05)
Negative mood	3.65 (1.84)	3.32 (1.53)	3.68 (2.00)	3.43 (2.00)	3.64 (1.80)	3.38 (1.63)	3.69 (2.01)	3.31 (1.78)
Urge	8.02 (1.69)	7.21 (2.27)	7.57 (2.46)	7.41 (1.98)	7.74 (1.85)	7.24 (2.13)	8.09 (2.11)	7.33 (2.28)

Note. Possible range = 0–10.

variant group comprised participants who were either heterozygous or homozygous for the Asp40 variant and the Asn40 group comprised those homozygous for the Asn40 variant. Participants were grouped by DRD4 status using conventional methods (Hutchison, McGeary, Smolen, Bryan, & Swift, 2002; Hutchison et al., 2003), with the DRD4-L group composed of individuals with at least one copy of the ≥7 repeat allele and the DRD4-S group composed of individuals who had neither copy greater than six repeats. The observed frequency of the DRD4 and OPRM1 genotype combinations were DRD4-S and OPRM1 Asn40 $n = 43$, DRD4-S and OPRM1 Asp40 $n = 21$, DRD4-L and OPRM1 Asp40 $n = 29$, and DRD4-L and OPRM1 Asp40 $n = 12$. The allele frequencies for the Asn40Asp SNP in this sample were in conformity with Hardy-Weinberg equilibrium expectations.

Data Analytic Plan

All analyses were performed using the SAS statistical package, Version 9.1. Variables were first checked for distributional assumptions. Group comparisons on demographic and other individual-difference measures were conducted using independent-samples *t* tests for continuous variables and chi-square tests for categorical variables (see Table 1). Generalized estimating equations (GEEs; Zeger, Liang, & Albert, 1988) were performed to examine the relationship between genotype and subjective responses to alcohol. The unit of analysis was participants' begin and end drink observations.

GEE models are essentially regression equations, either linear or logistic regression, that allow for varying numbers of observations per participant, while controlling for autocorrelation (we used the autoregressive AR1 structure; an exchangeable structure produced very similar results). Specifically, we used the GEE method to model the main effects of genotype, eBAC, and their interaction (at the subject level) on each of the dependent variables of interest (i.e., vigor, negative mood, and urge to drink), while controlling for begin drink report, as time-varying covariates. The GEE framework was most appropriate for this study because we were interested in between-subject factors (i.e., genes) as predictors of differences in mean levels of an outcome (i.e., subjective response to alcohol and urge to drink; Schwartz & Stone, 1998).

The following additional time-varying covariates were added, each separately, to the models to further probe for the genotype effects observed: (a) setting (i.e., where individuals were drinking), (b) whether or not others were drinking, and (c) time since last cigarette (among smokers only). Gene × Environment interactions were examined with the time-varying covariates. In light of the significant gender imbalance in the DRD4 and OPRM1 groups (shown in Table 1) and the higher frequency of female participants among consenters to DNA collection, gender was used as a covariate in all analyses. Analyses were repeated with smoking status (yes or no) in the model to explore its possible moderating effect on the hypothesized relationships.

Corrections for Type I error were considered but ultimately rejected based on the argument that Type I error needs to be considered for each hypothesis separately and not for the number of variables in the whole set of analyses reported (Dar, Serlin, & Omer, 1994). In the present analyses, no more than two measures assess a single hypothesis, thereby suggesting that corrections for Type I error may not be warranted.

Results

Analyses comparing consenters ($n = 112$) and nonconsenters ($n = 64$) on the baseline variables listed in Table 1 revealed that the two groups did not differ on several baseline characteristics, such as age, ethnicity, years of education, alcohol diagnosis, smoking status, and DrInC-2R scores (*p*s = *ns*). Nonconsenters were more likely to be male (47.1% vs. 21.6%), $\chi^2(1, N = 175) = 11.99$, $p < .0001$; had a higher average drinks per day, $t(173) = 2.46$, $p < .05$; and had higher drinks per drinking day, $t(173) = 2.44$, $p < .05$.

Drinking Episodes and Subjective Effects

Across the 5 days of prerandomization data collection for this study, there was a total of 262 begin Drink 1 reports, 259 end Drink 1 reports, 223 begin Drink 2 reports, and 223 end Drink 2 reports. This resulted in a total of 259 complete Drink 1 reports (begin Drink 1 + end Drink 1) and 223 complete Drink 2 reports (begin Drink 1 + end Drink 1 + end Drink 2). Only 36 drinking episodes consisted of a single drink, whereas the remaining 223 episodes consisted of two drinks. Participants reported an average of 2.31 drinking episodes over the 5-day assessment period. Morning report data for the 5-day period were culled for the purpose of this study and revealed that participants reported consuming an average of 4.67 ($SD = 5.45$) drinks per drinking episode, of which the first two drinks were captured via EMA assessments and represent the focus of this report.

The EMA design allowed us to capture contextual and environmental variables, such as setting, tobacco use, and whether or not others were drinking. With regard to setting, 47.3% of the drinking episodes occurred at the participant's home, 16.2% at someone else's home, 24.9% at a bar or restaurant, and 11.6% elsewhere. In 18.8% of the episodes, participants were alone, whereas, in 79.0% of the episodes, they were in the company of others and, in 2.2% of the episodes, others were "in view." More specifically, others were drinking in the participant's group on 71.3% of the episodes, others were drinking in the participants' view on 9.2% of the episodes, and participants were drinking alone in 19.5% of the episodes. Participants reported the presence of alcohol cues such as ads and seeing a liquor store, bar, or drinking place on 42.1% of episodes. In 79.3% of episodes, participants reported the presence of contextual alcohol cues, such as the people they drink with, place where they drink, time of day when they drink, day of week they drink, or other cues. Participants reported smoking a cigarette while drinking on 27.8% of episodes. Analyses revealed no significant differences in eBAC as a function of OPRM1 (GEE parameter estimate = −0.004, $SE = 0.003$, z score = −1.37, $p = .17$) or DRD4 (GEE parameter estimate = −0.003, $SE = 0.004$, z score = 0.83, $p = .41$) genotypes. There was, however, a significant association between gender and eBAC (GEE parameter estimate = 0.008, $SE = 0.002$, z score = 4.20, $p < .001$), suggesting higher eBAC for female participants.

Effects of OPRM1 Genotype

After drinking, carriers of the Asp40 allele reported higher vigor scores than homozygotes for the Asn40 allele after controlling for gender, eBAC, and vigor reported at the begin drink report (i.e., at baseline). OPRM1 genotype also had a significant main effect on

negative mood, after controlling for the covariates described above. Carriers of the Asp40 allele reported significantly lower levels of negative mood after drinking, as compared to homozygotes for the Asn40 variant. There were significant BAC × OPRM1 Genotype interactions on the mood variables, suggesting that although Asp40 carriers reported higher overall vigor and lower negative mood after drinking, as eBAC increased, carriers of the Asp40 allele reported greater decreases in vigor (see Figure 1) and greater increases in negative mood (see Figure 2), compared to individuals who were homozygous for the Asn40 allele (see Table 3).

To probe for whether the magnitude of changes in mood and urge to drink differed as a function of genotype differences at begin drink report, we added a Baseline × Genotype parameter to the models shown in Table 3. Results revealed a significant Baseline × Genotype interaction for negative mood (GEE parameter estimate = 0.34, $SE = 0.10$, z score = 3.33, $p < .01$), such that the relationship between begin drink report and end drink report for negative mood was stronger for carriers of the Asp40 allele than for Asn40 homozygotes. However, controlling for Baseline × Genotype interactions did not change any of the results reported in Table 3. Lastly, given that eBAC was significantly associated with gender, we controlled for the eBAC × Gender interaction in all of the models above. Results indicated that the eBAC × Gender term was not significant in any of the models above and that the addition of this parameter did not change any of the results reported in Table 3.

Effects of DRD4 Genotype

There was a significant main effect of DRD4 genotype on urge to drink and a significant eBAC × DRD4 Genotype interaction after controlling for the model covariates. Overall, carriers of the DRD4-L allele reported significantly greater urge to drink following alcohol consumption than individuals who were homozygous for the DRD4-S allele. The BAC × DRD4 Genotype interaction indicated that DRD4-S participants reported greater increases in urge to drink as BAC increased, as compared to DRD4-L individuals (see Figure 3). As with the OPRM1 genotype analyses described above, we added a Baseline × Genotype parameter to the DRD4 models shown in Table 3 and found no significant effects. Likewise, we added the eBAC × Gender term to each model and found that the results remained unchanged and that eBAC × Gender was only significant (GEE parameter estimate = −20.29, $SE = 8.37$, z score = −2.42, $p < .05$) when modeling negative mood. Specifically, male participants reported greater increases in negative mood at higher eBAC as compared to female participants.

Time-Varying Covariates

We examined the effects of three time-varying covariates, namely, (a) setting (i.e., location such as home, bar, restaurant, etc.), (b) whether or not others were drinking, and (c) time since last cigarette (among smokers only). Setting predicted self-reported vigor (GEE parameter estimate = 0.10, $SE = 0.05$, z score = 2.11, $p < .05$) such that participants reporter higher vigor when drinking in social settings. The addition of setting as a time-varying covariate did not significantly alter the results reported in Table 3, and there were no significant Gene × Environment interactions. Others' drinking was not associated with any of the dependent variables of interest. Time since last cigarette reduced the number of drinking episodes in the models to 135 given that 35.7% of the study sample ($n = 40$) were smokers. There was no significant effect of time since last cigarette on any

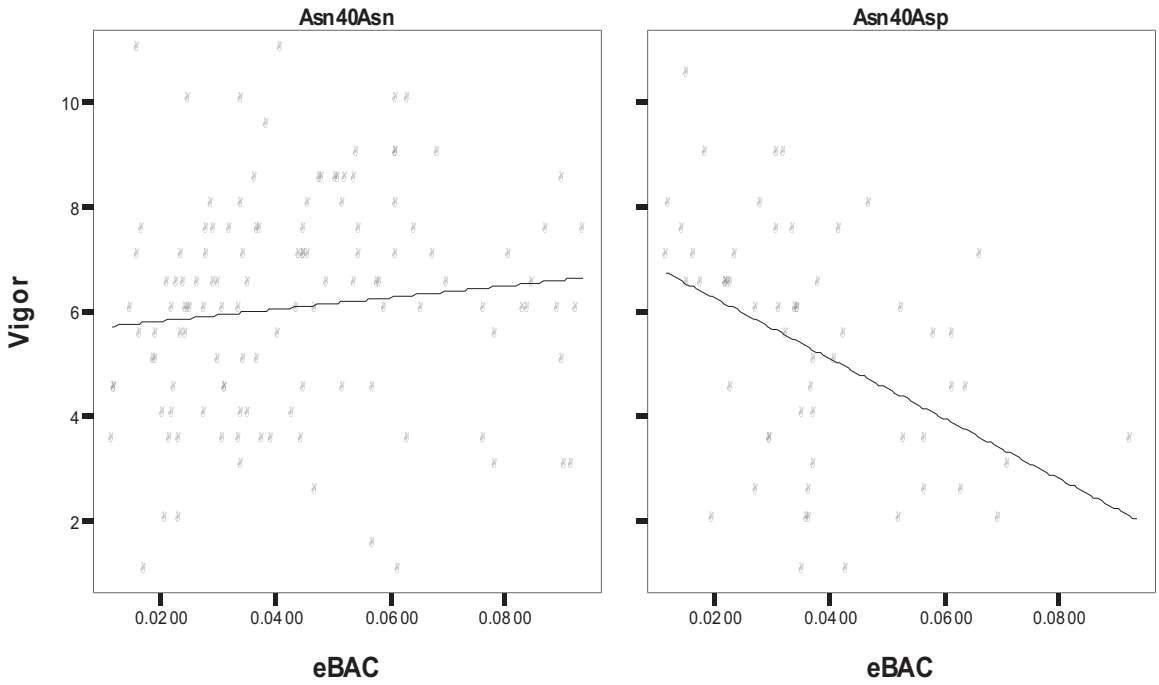

Figure 1. Vigor as a function of estimated blood alcohol concentration (eBAC) for individuals with the Asn40Asn and Asn40Asp genotypes of the OPRM1 gene.

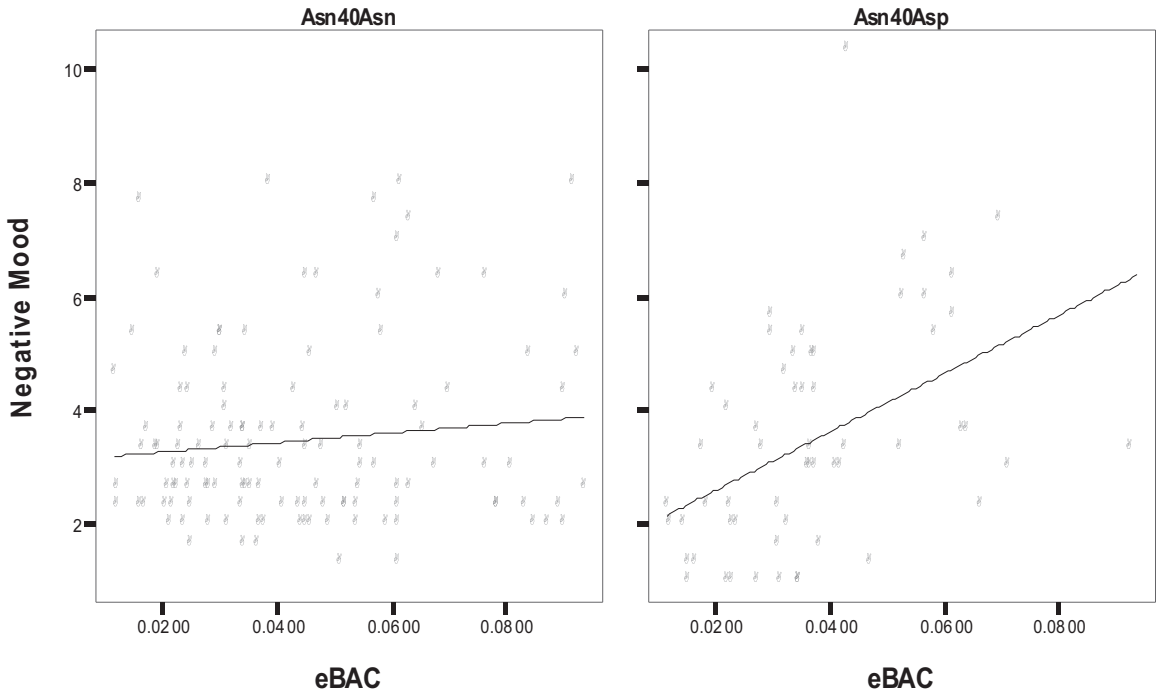

Figure 2. Negative mood as a function of estimated blood alcohol concentration (eBAC) for individuals with the Asn40Asn and Asn40Asp genotypes of the OPRM1 gene.

of the dependent variables of interest (*p*s = *ns*). GEE analyses in which smoking status (regular smoker: yes–no) was used to predict the dependent variables found no significant effects of smoking status (*p*s = *ns*) and did not significantly alter the results reported above.

Alcohol Consumption: Examining Morning Reports

We examined the relationship between subjective responses to alcohol and craving and drinking behavior using GEE models in which subjective responses and urge to drink at end drink report were predictors of the total number of drinks consumed during that episode, captured by the morning report. These analyses revealed that urge to drink (GEE parameter estimate = 0.44, $SE = 0.12$, z score = 3.74, $p < .001$) and vigor (GEE parameter estimate = 0.43, $SE = 0.15$, z score = 3.01, $p < .01$) were positively associated with alcohol consumption. Conversely, negative mood (GEE parameter estimate = −0.04, $SE = 0.21$, z score = −0.20, $p = .85$) was not significantly associated with alcohol consumption. These results suggest that vigor and urge to drink during the first two drinks, measured in the natural environment, predict subsequent alcohol consumption within that same drinking episode.

As a follow-up to the analyses of alcohol consumption assessed via the morning report, described above, we examined whether genotype (i.e., OPRM1 and DRD4, each tested separately) moderated the effects of vigor and urge to drink in determining alcohol consumption within an episode. GEE models were conducted in which alcohol consumption (captured via morning reports) was predicted by genotype, vigor (or urge to drink), and their interaction. Results revealed that alcohol consumption was predicted by urge to drink (GEE parameter estimate = 0.58, $SE = 0.15$, z score = 3.95, $p < .0001$), OPRM1 genotype (GEE parameter estimate = 4.73, $SE = 1.81$, z score = 2.62, $p < .01$), and their interaction (GEE parameter estimate = −0.66, $SE = 0.21$, z score = −3.08, $p < .01$). Specifically, greater urge to drink was associated with higher number of drinks, Asp40 carriers consumed a higher number of drinks, and urge to drink was less strongly associated with number of drinks consumed among carriers of the Asp40 allele within a given drinking episode. In short, these post hoc analyses suggest that urge to drink may be a less potent determinant of drinking behavior among Asp40 carriers. There was no other Genotype (i.e., OPRM1 or DRD4) × Subjective Response (i.e., vigor or urge to drink) interaction with regard to alcohol consumption assessed via morning report.

Discussion

In this study, we examined whether laboratory-based findings regarding genetic influences on subjective responses to alcohol and urge to drink generalize to the natural environment. To this end, heavy drinkers, 61% of whom were alcohol dependent, used handheld EDs to monitor drinking episodes for 5 consecutive days. Analyses revealed that carriers of the Asp40 allele of the OPRM1 gene reported greater feelings of vigor and less negative mood during drinking episodes, as compared to homozygotes for the Asn40 allele. This is generally consistent with the a priori hypotheses and laboratory-based findings of Ray and Hutchison (2004, 2007). Interestingly, the interactions between OPRM1 genotype and eBAC suggested that as BAC increased, carriers of the Asp40 allele reported greater decreases in vigor and greater increases in negative mood, compared to homozygotes for the Asn40

Table 3
Effects of OPRM1 and DRD4 Genotypes on Subjective Responses to Alcohol[1]

Model and predictor variables	OPRM1			DRD4		
	Parameter estimate (*SE*)	*Z*	*p*	Parameter estimate (*SE*)	*Z*	*p*
Vigor						
Genotype[a]	1.06 (0.51)	2.07	<.05	−0.34 (0.45)	−0.73	.47
eBAC	11.76 (6.33)	1.86	.06	−6.08 (8.16)	−0.74	.46
Genotype × eBAC	−41.87 (9.94)	−4.21	<.0001	8.08 (11.33)	0.71	.48
BA vigor	0.49 (0.07)	7.11	<.0001	0.52 (0.07)	7.40	<.0001
Gender	−0.27 (0.40)	−0.69	.49	−0.04 (0.41)	−0.11	.91
Negative mood						
Genotype[b]	−0.83 (0.32)	−2.61	<.01	0.46 (0.41)	1.13	.26
eBAC	−5.20 (4.56)	−1.14	.26	7.84 (9.39)	0.83	.40
Genotype × eBAC	22.46 (6.03)	3.73	<.001	−25.21 (12.32)	−2.05	<.05
BA negative mood	0.59 (0.07)	7.93	<.0001	0.61 (0.07)	8.44	<.0001
Gender	−0.05 (0.25)	−0.18	.86	0.04 (0.24)	0.16	.87
Urge to drink						
Genotype[c]	0.37 (0.50)	0.73	.46	0.89 (0.43)	2.07	<.05
eBAC	−1.47 (6.01)	−0.24	.81	9.56 (6.10)	1.57	.12
Genotype × eBAC	0.25 (13.88)	0.02	.99	−22.04 (10.73)	−2.05	<.05
BA urge to drink	0.47 (0.06)	7.51	<.0001	0.48 (0.06)	7.41	<.0001
Gender	0.35 (0.32)	1.09	.28	0.14 (0.27)	0.53	.59

Note. Apparent discrepancies between the means reported in Table 2 and the results reported in Table 3 are due to the fact that the begin drink report is the point of reference for Table 2, whereas the generalize estimation equation models use end drink report Data only, with baseline data as covariates. BA = baseline scores before each drinking episode (i.e., begin drink report), a time-varying covariate in all the models presented above; eBAC = estimated blood alcohol concentration.
[a] Carriers of the Asp40 allele scored higher than homozygotes for the Asn40 allele. [b] Carriers of the Asp40 allele scored lower than homozygotes for the Asn40 allele. [c] Carriers of the long allele scored higher than homozygotes for the short allele.

allele. These results suggest that the greater stimulant effects of alcohol reported by Asp40 carriers in their natural environment may be dose dependent and perhaps stronger at low levels of BAC.

The results supported the initial hypothesis that carriers of the long allele of the DRD4 gene would report greater urge to drink but offered no support for the notion that this polymorphism moderates subjective responses to alcohol. The finding that the DRD4-L allele was associated with greater urge to drink after alcohol consumption is consistent with previous laboratory studies (Hutchison et al., 2002; McGeary et al., 2006). However, an interaction between DRD4 genotype and eBAC indicated that at higher eBAC, urge to drink had a more pronounced increase among homozygotes for the short allele than carriers of the long allele. A recent study used fMRI to examine the neural correlates of these two polymorphisms upon presentation of alcohol taste cues and a priming dose of alcohol (Filbey et al., 2008). In this study, carriers of the long allele of the DRD4 VNTR had significantly greater neural response to alcohol taste cues (i.e., cue-exposure) in the orbitofrontal, cortex, anterior cingulate gyrus, and striatum prior to a priming dose of alcohol (i.e., cue-exposure) but not after a priming dose. These findings suggest that the effects of this polymorphism may be in response to alcohol cues and not necessarily the neuropharmacological effects of alcohol ingestion. While the present study cannot disentangle the effects of presence of alcohol cues from its pharmacology, it is consistent with the Filbey et al. (2008) results.

Conversely, the aforementioned imaging study revealed that Asp40 carriers had greater hemodynamic response in mesocorticolimbic areas both before and after a priming dose compared to homozygotes for the Asn40 allele (Filbey et al., 2008). Thus, the pharmacological effects of alcohol on endogenous opioids in the mesolimbic system (Erickson, 1996; Herz, 1997; Kreek, 1996) may be moderated by this polymorphism. These results are relevant to the literature showing that the Asn40Asp allele moderates the effects of naltrexone (Anton et al., 2008; McGeary et al., 2006; Oslin et al., 2003; Ray & Hutchison, 2007), a pharmacotherapy thought to dampen the reinforcing effects of alcohol (King, Volpicelli, Frazer, & O'Brien, 1997; Swift, Whelihan, Kusnetsov, Buongiorno, & Hsuing, 1994; Volpicelli, Watson, King, Sherman, & O'Brien, 1995). Additional studies and converging evidence from multiple lines of research (e.g., laboratory-based, clinical trials, EMA-based, neuroimaging) are necessary to more fully elucidate these complex mechanisms of genetic causation and their clinical implications to the etiology and treatment of alcohol use disorders.

Thus, for both the OPRM1 and DRD4 polymorphisms under study, participants' subjective responses to alcohol were more strongly dose dependent for carriers of the minor alleles (i.e., Asp40 and DRD4-L), such that these individuals reported overall greater levels of vigor, lower levels of negative mood (OPRM1 Asp40), and stronger urge to drink (DRD4-L) across drinking episodes. Nevertheless, at higher levels of eBAC, these individuals reported greater decreases in vigor, increases in negative mood, and lower increases in urge to drink, respectively. Further investigation on the nature of the OPRM1 (and DRD4 VNTR) × BAC interactions seems warranted to more fully elucidate the effects of these polymorphisms on subjective responses to alcohol and drinking behavior per se. This is particularly important considering that BAC was estimated and not directly measured in this study and

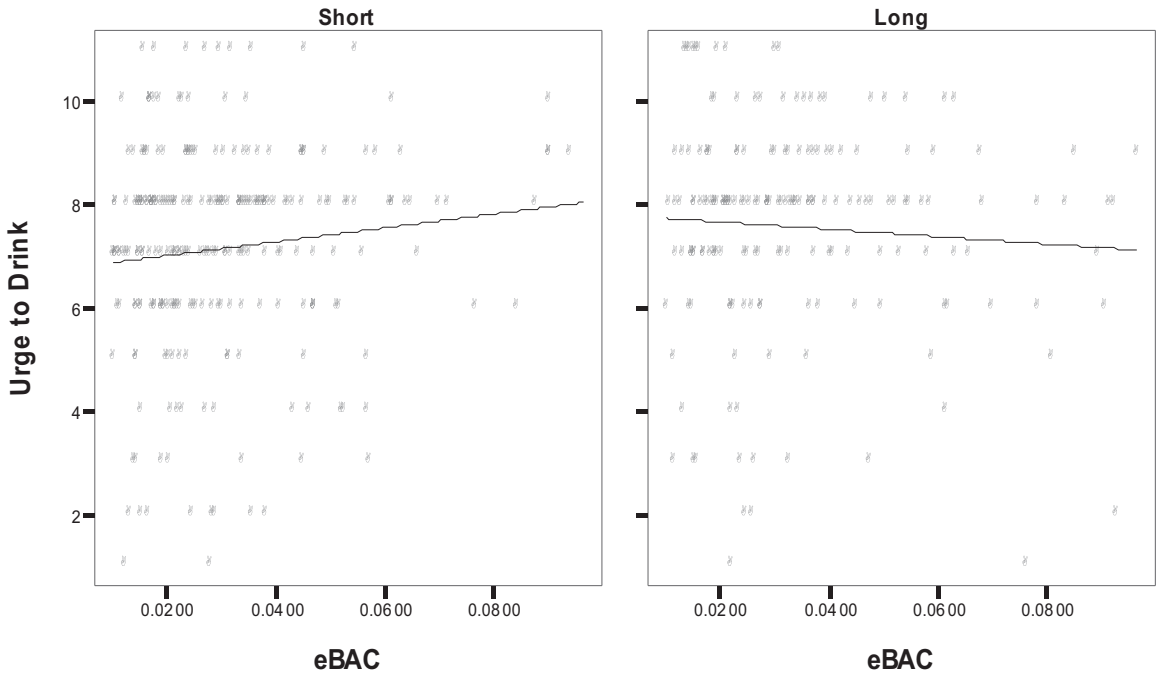

Figure 3. Urge to drink as a function of estimated blood alcohol concentration (eBAC) for individuals with the short and long alleles of the DRD4 gene.

that not all individuals reported drinking episodes at the various possible levels of BAC.

Interestingly, the pattern of OPRM1 Asn40Asp and DRD4 VNTR findings is consistent with Robinson and Berridge's (1993) incentive sensitization model of drug motivation. From this standpoint, the ascending corticomesolimbic dopamine circuit is largely responsible for attributions of incentive salience, or wanting, whereas opioidergic and other neurotransmitter systems variously subserve the hedonic impact, or liking, of both natural and drug rewards (Kelley & Berridge, 2002; Robinson & Berridge, 1993). Similarly, in the current study and other recent studies, functional genetic variation in the dopamine system has been associated with more pronounced craving (wanting) responses (e.g., Hutchison et al., 2002; McGeary et al., 2006; cf. van den Wildenberg, Janssen, et al., 2007), whereas functional genetic variation in the endogenous opioid system has been associated with variation in the psychoactive effects of alcohol (Ray & Hutchison, 2004, 2007). This is also consistent with the post hoc findings from this study suggesting that when controlling for urge to drink, carriers of the Asp40 allele drank 4.73 more drinks per episode than Asn40 homozygous and the OPRM1 × Urge to Drink interaction suggesting that urge to drink was less strongly associated with actual alcohol consumption among carriers of the Asp40 allele. The finding that urge to drink may be a less potent determinant of drinking behavior among carriers of the Asp40 allele is in line with the dissociation between wanting and liking, such that opioid-mediated processes are thought to be less strongly related to the former and more strongly associated with the latter. Despite this apparent consistency with the incentive sensitization approach and its extensive empirical basis, the literature on both of these polymorphisms remains relatively small, and the mechanisms underlying their relationship to alcohol use and misuse remain poorly understood.

Additionally, one may argue that tension-reduction or stress-response dampening models (e.g., Greeley & Oei, 1999; Levenson, Sher, Grossman, Newman, & Newlin, 1980; Sher & Levenson, 1982) may offer an alternative explanation of the current findings. Nevertheless, negative reinforcement assumes that the levels of negative mood have reached an unpleasant level and that the relief from negative mood results in negative reinforcement. That may be especially true in the case in of comorbidity between alcohol use disorders and mood and anxiety disorders, for instance. Conversely, if the levels of negative mood are at a normative level but are then lifted, or improved, by alcohol intake, then positive reinforcement is thought to occur. In other words, the current data do not allow us to determine how reinforcing these mood changes (i.e., vigor and negative mood) were to each individual. As reviewed by Sher, Grekin, and Williams (2005), the relationship between negative affective states and alcohol intake or problems is not a strong one, and laboratory-based studies have provided contradictory evidence on the effects of alcohol on negative affect. More specifically, the authors argued that negative affect regulation from drinking may be highly dependent upon intraindividual and situational factors, such as expectancies, genetics, and stressful environments (Sher et al., 2005). This is certainly as much of an empirical question as it is a theoretical one, and further research is needed to better understand the underlying structure of the various facets of subjective responses to alcohol, as well as their conceptual meaning and predictive utility. Of note, vigor and urge to drink upon consuming the first two alcoholic drinks were significantly positively associated with alcohol consumption within a given episode. These EMA-based findings offer a better under-

standing of the subjective responses that serve as antecedents, and perhaps determinants, of alcohol consumption in the natural environment.

Limitations of the study include the fact that the BAC estimation procedure was not as precise as that obtained in the laboratory and that these results may not generalize to treatment-seeking samples and/or social drinkers. In addition, selection bias in the group of consenters to the DNA analyses resulted in a greater representation of female participants among consenters. Although gender was controlled for in all genetic models, selection bias cannot be completely ruled out. Similarly, nonconsenters tended to be heavier drinkers than consenters, and as such, the selection of individuals with very heavy drinking patterns may bias the sample in terms of genetic and phenotypic characteristics related to responses to alcohol. In this study, only the initial two drinks of the day were assessed, which may not generalize to the subjective effects of alcohol observed at higher levels of BAC. Nevertheless, the subjective effects of alcohol after the first two drinks may be especially relevant to whether or not individuals escalate their drinking within a given episode and, more generally, in drinking situations. Lastly, the Gender × eBAC interaction was examined and accounted for in statistical models as well as in our procedures for estimating BAC. Nevertheless, not all individuals reported drinking episodes at the various levels of BAC, making it more difficult to fully evaluate the effects of gender in the present models.

Strengths include the study's external validity as it captures subjective responses to alcohol and urge to drink in nearly real-time in heavy drinkers' natural environment. The current study extends the literature on genetic factors underlying subjective responses to alcohol and drinking urges, constructs that have been typically studied under laboratory conditions and that are relevant to the etiology and treatment of alcohol abuse and dependence. Specifically, this study examined dimensions of subjective responses to alcohol in the natural environment (a) in the context of theory-driven genetic markers, (b) in relation to actual drinking during each episode, and (c) while considering important contextual time-varying covariates. Together, these methodological advantages afford a unique evaluation of subjective responses to alcohol and genetic markers that may underlie their expression. Similar to the distinction between efficacy and effectiveness trials, this study extended laboratory-based findings of the genetics of subjective responses to alcohol into real-world settings using EMA technology.

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