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ADAPTATION OF SUBJECTIVE RESPONSES TO ALCOHOL IS AFFECTED BY AN INTERACTION OF *GABRA2* GENOTYPE AND RECENT DRINKING

Ann E.K. Kosobud, Ph.D.[a], **Leah Wetherill, M.S.**[b,e], **Martin H. Plawecki, M.D., Ph.D.**[c], **David A. Kareken, Ph.D.**[a], **Tiebing Liang, Ph.D.**[d], **John L. Nurnberger, M.D., Ph.D.**[c], **Kyle Windisch, M.S.**[e], **Xiaoling Xuei, Ph.D.**[f], **Howard J. Edenberg, Ph.D.**[b,f], **Tatiana M. Foroud, Ph.D.**[b], and **Sean J. O'Connor, M.D.**[c,g]

[a]Department of Neurology, Indiana University School of Medicine, Indianapolis, IN, USA

[b]Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA

[c]Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN, USA

[d]Department of Medicine, Division of Endocrinology, Indiana University School of Medicine, Indianapolis, IN, USA

[e]Department of Psychology, Indiana University Purdue University at Indianapolis, Indianapolis, IN, USA

[f]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN, USA

[g]R.L. Roudebush Veterans Administration Medical Center, Indianapolis, IN, USA

Abstract

Background—Subjective perceptions of alcohol intoxication are associated with altered risk for alcohol abuse and dependence. Acute adaptation of these perceptions may influence such risk, and may involve genes associated with pleasant perceptions or the relief of anxiety. This study assessed the effect of variation in the $GABA_A$ receptor genes *GABRG1* and *GABRA2* and recent drinking history on the acute adaptation of subjective responses to alcohol.

Methods—132 non-dependent moderate to heavy drinkers, aged 21–27, participated in 2 single-blind, counterbalanced sessions, approximately one week apart. One session was an intravenous alcohol "clamp", during which breath alcohol concentration was held steady at 60 mg/dL (60 mg %) for 3 hours, and the other an identical session using saline infusion. Subjective perceptions of intoxication, enjoyment, stimulation, relaxation, anxiety, tiredness and estimated number of drinks were acquired before (baseline), and during the first and final 45 minutes of the clamp. A placebo-adjusted index of the subject's acute adaptation to alcohol was calculated for each of the 7 subjective measures, and used in a principal component analysis to create a single aggregate

Corresponding Author: Sean O'Connor, M.D., Professor of Psychiatry, Indiana University School of Medicine, Institute for Psychiatric Research, 320 West 15th Street Indianapolis, Suite 200, Indianapolis, IN, 46202, Phone: (317)274-1362, Fax: (317)274-1365, oconnor1@iupui.edu.

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estimate for each subject's adaptive response to alcohol. Analysis of covariance tested if *GABRA2* and *GABRG1* single nucleotide polymorphism (SNP) genotypes, gender, placebo session, family history of alcoholism, recent drinking history, and the genotype x recent drinking history interaction significantly predicted the adaptive response.

Results—Recent drinking history (p=0.01), and recent drinking history x genotype interaction (p=0.01) were significantly associated with acute adaptation of the subjective responses to alcohol for the *GABRA2* SNP rs279858.

Conclusion—Higher recent drinking was found to be associated with reduced acute tolerance to positive, stimulating effects of alcohol in carriers of the rs279858 risk allele. We postulate that the *GABRA2* effect on alcohol dependence may, in part, be due to its effect on subjective responses to alcohol.

Keywords

Intravenous alcohol; GABA receptors; Acute tolerance; Family history of alcoholism; Alcohol use disorders

INTRODUCTION

Whether alcohol intoxication is perceived as pleasant and/or aversive is likely to influence an individual's alcohol consumption. How subjective responses contribute to alcohol use disorders remains an area of active research (King et al., 2011; Quinn and Fromme, 2011a; Ray and Hutchison, 2009; Schuckit et al., 2012; Schuckit and Smith, 2013). Finding that sons of alcoholics consistently reported weaker subjective effects of alcohol on a number of measures (Schuckit, 1980; Schuckit, 1984), Schuckit proposed that individuals with a low level of response to alcohol were at higher risk for alcohol use disorders. Additional studies confirmed this association, and in particular low level of response to negative, sedating effects of alcohol appeared to predict heavier drinking and drinking problems (Schuckit et al., 2009; Schuckit et al., 2011; Schuckit and Smith, 2013; Trim et al., 2009).

However, a number of authors have reported heavier drinking associated with greater stimulant effects of alcohol, both in children of alcoholics (Erblich and Earleywine, 2003) and in the general population (Erblich and Earleywine, 2003; Holdstock et al., 2000; King et al., 2002). Newlin and Thomson (Newlin and Thomson, 1990) proposed that a higher risk for alcohol problems would be found in individuals who perceive enhanced stimulation during the absorption phase, when breath alcohol concentrations (BrAC) are rising, and reduced sedation during the alcohol elimination phase, when BrAC are falling. King *et al.* (King et al., 2011; King et al., 2014) confirmed greater stimulation during rise, and greater sedation during fall of BrAC in heavy compared to light social drinkers. But these investigators also found that heavy social drinkers had higher ratings of stimulation at peak BrAC, and reported greater liking and wanting of alcohol throughout the BrAC curve. In addition, the heavy drinkers who reported the most stimulation, liking, and wanting, and the least sedation were found to drink more, report the highest rates of alcohol abuse and dependence (King et al., 2011), and have the greatest risk for future alcohol use disorders

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(King et al., 2014). Thus, there appears to be consensus that both reduced sedation and enhanced stimulation are associated with risk of alcohol use disorders.

Studies of subjective responses during rising and falling limbs of the BrAC curve are complicated when oral dosing is used because individuals show significant variation in rise, peak and fall of BrAC (Ramchandani et al., 1999). Intravenous (IV) infusion of alcohol, based on an individual's physiologically-based pharmacokinetic parameters, assures identical BrAC trajectories among subjects by circumventing absorption kinetics and compensating for individual variation in distribution and elimination kinetics (Plawecki et al., 2008; Ramchandani et al., 1999). The improved control of BrAC available with IV infusion may provide better resolution of how subjective effects are affected by BrAC trajectories. Using IV infusion, our lab was able to measure subjective perceptions in subjects at an identical BrAC and time elapsed while BrAC was either rising or falling at prescribed rates of change (Wetherill et al., 2012). Subjects with recent moderate social drinking (mean and SEM 2.4±0.2 drinking days in the past 7 days) reported greater feelings of *intoxicated* and *high* on the ascending compared to descending slope, while recent light social drinkers (mean and SEM 1.9 ±0.4 drinking days in the past 7 days) reported the opposite; greater feelings of *intoxication* and *high* on the descending slope. Under these conditions neither group reported greater sedation on the descending slope.

In addition to the effects of rate of BrAC change, subjective responses to alcohol are known to adapt over time. The development of chronic tolerance to alcohol is one of the hallmarks of increased severity of an alcohol use disorder (AUD). To the degree that the capacity for developing chronic tolerance is reflected in acute, i.e. within-session, tolerance, the phenomenon can be studied in laboratory paradigms that assess changes in alcohol effects during one exposure to alcohol. One approach for assessing acute tolerance to alcohol, attributed to Mellanby (Mellanby, 1919, as described in Martin and Moss, 1993) is to measure some assay of alcohol's effects on the ascending limb of exposure, and at the same blood alcohol concentration on the descending limb, with differences in the magnitude of the effect attributed to acute tolerance or acute sensitization. For example, Portans (Portans et al., 1989) inferred acute tolerance to alcohol when subjects reported greater subjective effects on the ascending limb of the BrAC curve, compared to effects at a comparable BrAC on the descending limb. Alternatively, the magnitude of the effect at a particular blood alcohol concentration on the ascending limb is tracked until the effect is of the same magnitude on the descending limb, with differences in the blood alcohol concentration used as an index of acute adaptation to alcohol. In either approach, any brain sensitivity to the rate of change of alcohol exposure represents a confound that is compounded by the variability ensuing from use of the oral route of administration.

Using IV infusion to "clamp" BrAC at a specific concentration over an extended period is ideal for examining acute adaptation (acute sensitization or tolerance) to alcohol without the complications of intersubject variability in BrAC, or sensitivity to the rate or the direction of change (O'Connor et al., 1998). BrAC closely approximates the arterial alcohol concentration (Gomez et al., 2012), and therefore the brain's exposure to alcohol, since the brain is a high-flow, low-volume organ.

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Our lab has investigated whether recent drinking history or family history of alcohol are associated with differences in adaptation of subjective responses to alcohol during a 2 hour 60 mg/dL BrAC clamp. One report from this study documented an association of heavier recent drinking history with lower initial response and less acute tolerance as reflected by subjective ratings of *intoxication* and *high* (Ramchandani et al., 2002). A second report documented an association of a positive family history with higher initial subjective ratings of *intoxication*, and greater acute tolerance (Morzorati et al., 2002). Family history positive and negative subjects did not differ in recent drinking history (Ramchandani et al., 2002).

Differences in subjective responses due to family history suggest a role for genes. Genes encoding ionotropic gamma-aminobutyric acid ($GABA_A$) receptors are associated with alcohol use disorders (Borghese and Harris, 2012; Covault et al., 2008; Edenberg et al., 2004). SNPs in *GABRG1* and *GABRA2* have also been associated with various subjective responses to alcohol (Arias et al., 2014; Kareken et al., 2010; Pierucci-Lagha et al., 2005; Ray and Hutchison, 2009; Uhart et al., 2012), and to cues associated with alcohol intoxication (Kareken et al., 2010). Roh et al. used IV alcohol to hold BrAC steady at 50 mg/dL for 165 minutes in 110 moderate-heavy social drinkers and found that adaptive subjective responses to alcohol were affected by a combination of *GABRA2* and *ALDH2* SNP status (Roh et al., 2011).

In the present study, seven subjective responses to alcohol were measured prior to and during a 3-hour IV alcohol infusion during which BrAC was held steady at 60 mg/dL; a placebo, saline infusion, session was also conducted in counterbalanced order. We hypothesized that alleles in *GABRA2* and *GABRG1* previously shown to be associated with alcohol-related risk phenotypes would also be associated with acute adaptation of the subjective response to alcohol during the clamped steady-state interval. We also tested if this association was affected by recent drinking history, family history of alcoholism, or gender.

METHODS

Subjects

Social drinkers, ages 21 to 27 years, were recruited through local advertisement and then screened by telephone for general eligibility. The sample was limited to European Americans to reduce the potential confounds introduced by SNPs whose allele frequencies differ across racial groups (Cross et al., 2010; Ittiwut et al., 2008). Those who passed the phone screen were invited to the laboratory for a detailed interview which began with a measurement of BrAC to confirm sobriety, followed by acquisition of informed consent approved by the Indiana University School of Medicine Institutional Review Board.

All interviews and testing were conducted at the Indiana Clinical Research Center by trained research technicians. Inclusion criteria were good health as determined by physical exam, a blood test of liver function indices, and subject-reported medical history and consumption of at least 17 drinks in the past month. Exclusion criteria, all based upon subject reports, included current or prior history of any serious disease (including central nervous system, cardiovascular, respiratory, gastrointestinal, hepatic, renal, or endocrine), positive hepatitis or HIV test, alcoholism in the biological mother during pregnancy (to minimize potential

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effects of Fetal Alcohol Syndrome Disorder), current or prior history of severe alcohol-induced flushing reactions, current or prior history of Axis-I psychiatric illness including alcohol or drug dependence (but not alcohol abuse) assessed using the Semi-Structured Assessment of the Genetics of Alcoholism (SSAGA) (Bucholz et al., 1994), use of medications known to interact with alcohol within 2 weeks of the study; and females who were or intended to become pregnant. In addition, subjects were excluded if, on the day of testing, they had a positive BrAC, presence of illicit drugs on urine drug screen, or, for females, a positive urine pregnancy (hCG) test.

Family history of alcoholism (FHA) was assessed using the Family History Assessment Module (FHAM) (Rice et al., 1995). Family history of alcoholism was defined as positive if: 1) one 1^{st} degree, and 2) at least another 1^{st} or 2^{nd} degree biological relative was reported to have alcohol dependence (lifetime). Family history of alcoholism was defined as negative if no 1^{st} or 2^{nd} degree relatives were reported to have alcohol dependence (lifetime). Subjects not meeting definitions either for family history positive or negative were excluded from the study. Recent drinking history was assessed for the 30 days prior to the interview using a Timeline Follow-Back (TLFB) (Sobell et al., 1988). Four measures were derived from the TLFB (drinking days, drinks per drinking day, heavy drinking days, and total drinks). Subjects were asked about both the volume and type of alcohol they consumed, and their responses used to estimate the number of standard drinks (12 g of ethanol) recorded in their TLFB.

Genotyping

DNA was extracted from frozen whole blood samples using the Puregene Genomic DNA Purification Kit from QIAGEN (catalog #158489, Valencia, CA, USA) following the manufacturer's instructions. Based on previous reports of association with alcohol-related phenotypes and on linkage disequilibrium in this region, one SNP in *GABRG1* (rs1497577) and one SNP in *GABRA2* (rs279858) (r^2=0.18) were genotyped using iPLEX Gold assays designed with MassArray Assay Design Software (Sequenom, San Diego, CA). SNP information is shown in Table 1.

General procedure

Each subject participated in two single-blind, counterbalanced-order study days. Each subject received an infusion of 6% ethanol in half-normal saline during one session and a placebo infusion of half-normal saline at a comparable infusion rate profile during the other. Subjects were advised that they would receive alcohol during one or both visits but they were not told which study day was an alcohol day. On each study day, the subject arrived at the laboratory by 7:00 AM, and underwent a brief physical exam, as well as breathalyzer and urine testing. Then the subject was offered a 550 calorie breakfast, after which a 20 gauge indwelling venous catheter was placed in an antecubital vein of each arm, flushed with saline and capped with a heparin lock. Subjects were also fitted with a scalp electroencephalography cap and heart rate leads (data not presented here). The infusion session began at approximately 10:15 AM.

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Testing in each session comprised three 45 min blocks during which multiple tasks were administered in the same order (Figure 1); only results for subjective perceptions are reported here. Block 0 (Baseline) was obtained just prior to the infusion; subjects were aware that they had not received any alcohol at this point in the protocol. The alcohol or placebo infusion was then initiated, and Block 1 began either 20 min later (placebo session) or after the 60 mg/dL alcohol clamp was established and stable for 5 minutes (19.4 ± 0.04 min (mean ± SEM)). Block 2 began 105 min after the start of Block 1.

Management of the Infusion rate profile

The infusate was prepared by the Indiana University Hospital research pharmacy. Infusion rates were computed and delivered by our Computer-assisted Alcohol infusion System (CAIS, Zimmermann et al., 2008; Plawecki et al. 2012, 2013). An individualized infusion rate profile was pre-calculated using a transformation of the subject's age, height, weight and gender into the parameters of a physiologically-based pharmacokinetic model of alcohol distribution and elimination (O'Connor et al., 1998; Plawecki et al., 2008; Ramchandani et al., 1999). A computerized algorithm generated an infusion rate profile that, when coupled with automated adjustments based on real-time BrAC measurements, achieved induction and maintenance of a fixed BrAC of 60.0 ± 0.2 mg/dL (Mean ± SEM) from the beginning of Block 1 to the end of Block 2. BrAC measurements were taken with an Alcotest meter, model 7410 or 6510 (Draeger, Irving, Texas).

Measurement of Subjective Responses to Alcohol

Prior to Block 0, subjects were familiarized with the battery of tasks in a practice block consisting of abbreviated versions of all the tasks except for the subjective perceptions test, which was administered exactly as it would be in Blocks 0, 1 and 2. Except for bathroom breaks, subjects remained seated in the testing booth; they were instructed to bring an activity of their choice to pursue while tests were not being administered. Most subjects brought books or laptops; cellphone use was allowed.

Numerical ratings of subjective perceptions were acquired at the beginning and end of each block; the two scores were averaged to give a single score for each block. Subjects responded to 7 questions using a computerized visual analog scale (Figure 2), as implemented in past studies (Morzorati et al., 2002; Ramchandani et al., 2002; Wetherill et al., 2012). Six of the 7 questions included a term followed by several dictionary-derived synonyms intended to provide subjects with a common understanding. These questions were designed to encompass a broad range of possible subjective effects of alcohol: Tired (and its synonyms) reflected negative sedation; Relaxed (and its synonyms): positive sedation; Anxious: negative stimulation; Stimulated: positive stimulation; Enjoying: liking of the alcohol experience; Intoxication: feeling a drug effect. Estimated Number of Drinks was included as an intuitive metric reflecting the subject's prior experience with alcohol.

Calculation of Initial and Adaptive subjective responses

For each subjective measure, initial and adaptive responses were calculated for both the placebo and alcohol sessions. The initial response was computed as Block 1 score – Block 0 score, so that a positive value indicated an increase from baseline, and a negative value

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indicated a decrease. A single, scalar adaptive index for each subjective response was derived from the data from Block 0, 1 and 2: acute adaptation = Sign(B1−B0)*(B2−B1). The Sign function allowed positive values of the index to be interpreted as acute sensitization and negative values as acute tolerance to alcohol. Then, the adaptive indices for the placebo session were subtracted from the respective adaptive indices for the alcohol session to create for each subject a placebo-adjusted adaptive response index.

Statistical Analysis

Analyses were conducted in SAS v9.3. To reduce the burden of multiple measures testing, a principal components analysis was used to combine placebo-adjusted indices for the 7 subjective measures into a smaller number of factors. Principal component, rather than exploratory factor analysis, was employed as the recommended approach for data reduction and in order to maximize the total variance accounted for, as no underlying latent construct was hypothesized for this variable set (Fabrigar, 1999; Brown, 2000). Principal components accounting for at least 20% of the variability in the aggregate measure were included as potential phenotypes of interest. Potential confounds due to differences in demographic variables were assessed. Chi-squared tests assessed differences between pairs of categorical demographic variables (family history of alcoholism, gender, session order). Mantel-Haenszel tests were used to test if categorical demographic variables differed by SNP genotype. T-tests were employed to test if drinks per drinking day and age differed by gender or family history of alcoholism. Analysis of variance tested for the additive effects of each SNP genotype on drinks per drinking day and age.

Analysis of covariance (ANCOVA) models analyzed the principal component score for the placebo-adjusted adaptive response as the dependent variable, with family history of alcoholism, recent drinking history, and SNP genotype as potential main effects. Gender was included as a covariate. Due to the *a priori* hypothesis that family history, recent drinking history and genotype variation would contribute to the adaptive response, all main effects were included in the final model. All 2-way interactions between the main effects and gender were tested. Other interactions and covariates were included in the final model only if significant ($p<0.05$). Separate analyses were performed for each SNP, modeling genotype as an additive effect.

RESULTS

141 subjects completed both infusion sessions; of these, 132 had complete phenotype and genotype data and are included in the analysis. The numbers of subjects within subgroups in the final sample are included in Table 1. Drinks per drinking day averaged 5.0±0.2 overall (minimum 1.5, maximum 13.9) reflecting moderate to high-risk, but non-dependent, drinking. Mean and SEM drinks per drinking day for subgroups are shown in Figure 3; only gender was significant (Females<Males, $p=0.001$). For the alcohol clamp, the average time for a linear ascent to 60 mg/dL was 14.4 ± 0.04 min (Mean ± SEM). BrAC measurements obtained during Block 1 and Block 2 averaged (± SEM) 60.4 ± 0.23 and 60.3 ± 0.11 mg/dL, respectively.

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Figure 4A shows the initial response to alcohol or placebo. For the first 5 subjective measures shown, the initial response to alcohol was an increase while for tiredness, a decrease was observed. About half of the subjects rated anxiety zero at all time points, and of the remainder, roughly equal numbers of subjects reported increases as reported decreases. During the placebo infusion, mean responses were in a similar direction as those observed during the alcohol infusion, but were much smaller. The perceived number of drinks dropped by approximately 1. Figure 4B shows the adaptive response. All 7 subjective measures showed acute tolerance to alcohol infusion (i.e., negative values, indicating a return toward baseline), regardless of the direction of the initial response. For the placebo infusion, the direction of the adaptive response indicated acute tolerance for all measures except tiredness, which showed acute sensitization; again, much smaller subjective responses were reported during placebo infusions.

Table 2 shows correlation coefficients between pairs of the 7 subjective measures, calculated using the placebo-adjusted adaptive response. Table 3 shows the results of the principal component analysis performed using these measures. The first principal component (PC1) for the placebo-adjusted adaptive response had an eigenvalue = 2.52, and accounted for ≥ 36% of the observed total variance. The variables with the highest correlation coefficients, Enjoyment, Intoxication, Stimulation, and Number of Drinks were the strongest contributors to PC1. The second principal component (PC2) had an eigenvalue = 1.2, but accounted for less than 18% of the variability, and was not used in subsequent analyses.

Males reported significantly more drinks per drinking day (5.6±0.3) compared to females (4.3±0.2; $p=0.001$); therefore gender was included as a covariate in the primary analyses of PC1 for acute adaptation. There were no significant differences between any other pairs of demographic variables (all $p\geq0.10$). There was no significant effect of session order on PC1 ($p>0.49$); hence session order was excluded from further analyses. The final model included family history of alcoholism, recent drinking history, SNP genotype, and the recent drinking history*genotype interaction; gender was retained as a covariate.

Table 4 shows the ANCOVA results for the two SNPS tested. The model testing the acute adaptive response to alcohol that included the *GABRA2* SNP rs279858 found recent drinking history was associated with acute tolerance ($p=0.01$). In addition, there was a recent drinking history x genotype interaction ($p=0.01$). The model that included the *GABRG1* SNP rs1497577 demonstrated no significant effects (all $p>0.13$).

To illustrate the recent drinking history x genotype interaction observed for the *GABRA2* SNP, a median split (median = 4.6 drinks/drinking day) was used to assign subjects to either a higher or lower recent drinking history group. As shown in Figure 5, all groups had negative average PC1 scores, indicating acute tolerance to the subjective effects of alcohol that comprise PC1, but they differed in degree. In the higher recent drinking history group, individuals with the CC genotype showed the least acute tolerance and individuals with the TT genotype showed the greatest acute tolerance. In the lower recent drinking history group, the opposite was observed: individuals with the CC genotype displayed the greatest acute tolerance while subjects with the TT genotype displayed less tolerance. Individuals with the

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CT genotype displayed intermediate PC1 values in the higher drinking group, and resembled TT individuals in the lower drinking group.

DISCUSSION

Our data show that for all 7 subjective measures, acute tolerance to alcohol developed within 3 hours when BrAC was held at 60 mg/dL. The placebo-adjusted adaptive responses calculated for each of the 7 measures were combined into a single index, PC1, using principal components analysis. PC1 was tested for the effects of SNPs in *GABRA2* and *GABRG1*. The model that included rs279858 (in *GABRA2)* found recent drinking history was associated with acute tolerance (p=0.01). Although that SNP itself showed only a trend toward significance, there was a significant recent drinking history x genotype interaction (p=0.01). Among individuals who reported higher levels of drinking in the preceding 30 days, those carrying the T allele showed more tolerance than high-drinking individuals with the CC genotype. Conversely, among individuals who reported lower drinking in the preceding 30 days, those carrying the T allele showed less tolerance than those with the CC genotype (Figure 4). The C allele of this SNP has been associated with alcohol dependence (Fehr et al., 2008, Covault et al., 2008). The model that included rs1497577 (in *GABRG1*) demonstrated no such association.

PC1 was mostly comprised of pleasant effects such as enjoyment and stimulation (defined for subjects as positive stimulation by use of the synonyms lively, up, vigorous and excited), as well as intoxication and estimated number of drinks. The negative subjective effects (anxiety, tiredness) contributed less to PC1. This dichotomy suggests that for heavier drinking individuals who carry the CC genotype at rs279858, the pleasant effects of alcohol persist within an episode of drinking to a greater degree than in lighter drinking CC individuals. These persisting pleasant effects of alcohol could contribute to longer drinking episodes, and so to an increased risk of alcohol use disorders. While our study only assessed recent drinking patterns (within 1–2 months of participation), our finding is consistent with a number of studies showing that recent or extended heavy drinking history is associated with greater positive and stimulating effects of alcohol (i.e. measures such as stimulate, like, want, enjoy) (King et al., 2011; King et al., 2014; Quinn and Fromme, 2011b).

Only one recent study has looked at the acute adaptive response to alcohol. Using BrAC clamping, Roh et al. (Roh et al., 2011) tested the association of 6 *GABRA2* SNPs with subjective responses to alcohol in a sample of Japanese adults. These authors found that in individuals with the alcohol dehydrogenase gene *ALDH2*1/*1*, the *GABRA2* SNP rs279837 was associated with differences in acute adaptation to alcohol (Sensation Scale dynamic-peripheral, BAES stimulant, and BAES sedative subscales; direction of difference not reported). But no significant effects were found for our SNP (rs279858) or for the other 5 *GABRA2* SNPs tested. This lack of consistency may be due to the difference in subject populations, and in our use of recent drinking history as a cofactor. Other differences between Roh et al. and the present study include the proportion of family history positive individuals (2.6% vs 50%, respectively), different BrAC levels of intravenous clamping (50 mg/dL vs. 60 mg/dL), different subjective scales, number of items, content and number of times given; and differences in the calculation of acute adaptation.

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Several investigators have examined the role of *GABRA2* in various subjective responses to alcohol, although most have not used the alcohol clamp or looked at acute adaptation. Pierucci-Lagha et al.(2005) found reduced stimulation in response to oral alcohol in subjects carrying one or two copies of the rs279858 alcohol dependence-associated risk allele, but no difference in sedative effects. Uhart et al.(2012), comparing oral alcohol to a placebo, found that subjects with 1 or 2 copies of the risk allele or a correlated allele for 6 *GABRA2* SNPS, including the C allele for rs279858, were less likely than the alternate allele homozygotes to report negative effects of alcohol such as “Disliked”, felt “Bad”, or felt “Worst Ever”. However, these investigators also used two other subjective scales (the BAES (Biphasic Alcohol Response Scale), and items from Schuckit’s SHAS (Subjective High Assessment Scale), and found no genetic associations with negative or positive subjective effects of alcohol. Finally, Arias and colleagues (2014), also using oral alcohol administration, found that subjects with one or two copies of the rs279858 C risk allele reported greater stimulation on the BAES, and higher “like alcohol”, “feel ‘high”, and “feel alcohol” on the Drug Effects Questionnaire, compared to subjects homozygous for the TT allele. Thus, while investigators frequently find that *GABRA2* SNPs are associated with differences in subjective responses to alcohol, the reports are inconsistent; such contradictions might be due to variation in individual alcohol exposures after oral alcohol administration (Ramchandani et al., 1999), or to differences in type and number of scales used and when, how, and how often they were administered.

The present study found no association of family history of alcoholism with the overall measure of acute adaptation. This is surprising, as family history would be expected to capture greater genetic variation in individual responses to alcohol than a single SNP, and multiple studies have shown that subjective responses to alcohol differ in individuals with a history of alcoholism in their families compared to subjects with no family history (e.g. Morzorati et al., 2002; Schuckit and Smith, 1996) though several studies have not (e.g. (Kerfoot et al., 2013; Wetherill et al., 2012). It is possible that in the present study, the genotypic effects of *GABRA2* accounted for much of the variability in the acute adaptation of subjective perceptions, and that family history did not account for a substantial portion of the remaining variability.

Interpretation of our findings is subject to limitations. In particular, a recent paper describing an attempt to replicate studies of candidate genes influencing subjective responses to amphetamine (Hart et al., 2013) indicates a high probability of false positives, due to factors including insufficient power due to small numbers of subjects (particularly when a rare homozygote is present); publication bias; failure to correct for multiple testing (especially correction for non-published, non-significant tests) and use of intermediate phenotypes, like subjective responses, that are themselves highly variable within and among subjects. Our use of principal components to combine multiple subjective measures into a single component reduced the number of statistical tests necessary; and may have provided us with a less variable indicator of subjective phenotype, but more work is necessary to evaluate the merits of this approach.

The sample population was limited to European Americans and young adults. Recent drinking history was derived from a 30 day TLFB taken at time of enrollment; no

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assessment of longer term drinking history was included. A double-blind study was not possible due to the several BrAC measurements taken during the alcohol infusion that are employed as real-time feedback to the software computing individual infusion rate profiles in order to insure identical trajectories of alcohol exposure. However, the quality of the single-blind was maintained by acquiring BrAC readings with the same frequency during the placebo infusion as during the alcohol infusion, and the subject was not informed of the BrAC results in either session. In addition, session order (placebo vs alcohol in the first session) was not significant, and responses during the placebo session were accounted for in the statistical model.

Another limitation is the use of intravenous infusion of alcohol rather than oral ingestion. That limitation is minimal in the context of alcohol effects on brain function, per se. In addition, the IV infusion eliminates an unknown degree of individual variability in responses attributable due to taste, odor, and beverage preference in oral studies. It also virtually eliminates the substantial variation in the trajectories of brain exposure attributable to uncontrollable alcohol absorption kinetics (Ramchandani et al., 2006) that are inherent in all oral studies; oral administration cannot achieve and sustain a predetermined level of BrAC because of those absorption kinetics.

Finally, the sample population was limited to non-dependent drinkers; it is possible that this group includes survivors of risk, and the observed associations reflect protective effects rather than risk. Nonetheless, we believe that demonstration of an association of *GABRA2* and recent drinking with acute adaptation to alcohol, at an exposure frequently achieved in social drinking, is informative in regard to differential risk for development of alcohol use disorders.

In summary, we have shown that a polymorphism in *GABRA2* interacting with recent drinking history is associated with the acute adaptation to steady-state alcohol intoxication. The measure of adaptation included as components the subjective responses of intoxication, enjoyment, number of drinks, stimulation, relaxation, anxiety, and tiredness as achieved with intravenous administration. It is possible that for individuals who are homozygous for the C allele, heavy drinking leads to a more positive drinking experience, leading to a greater risk for developing an alcohol use disorder. Further analyses are warranted not only to replicate these findings, but also to evaluate their predictive value in anticipating problem drinking, and whether they can be extended to subjects of other ethnic background. If confirmed, these results will aid in understanding the complex mechanisms contributing alcohol dependence.

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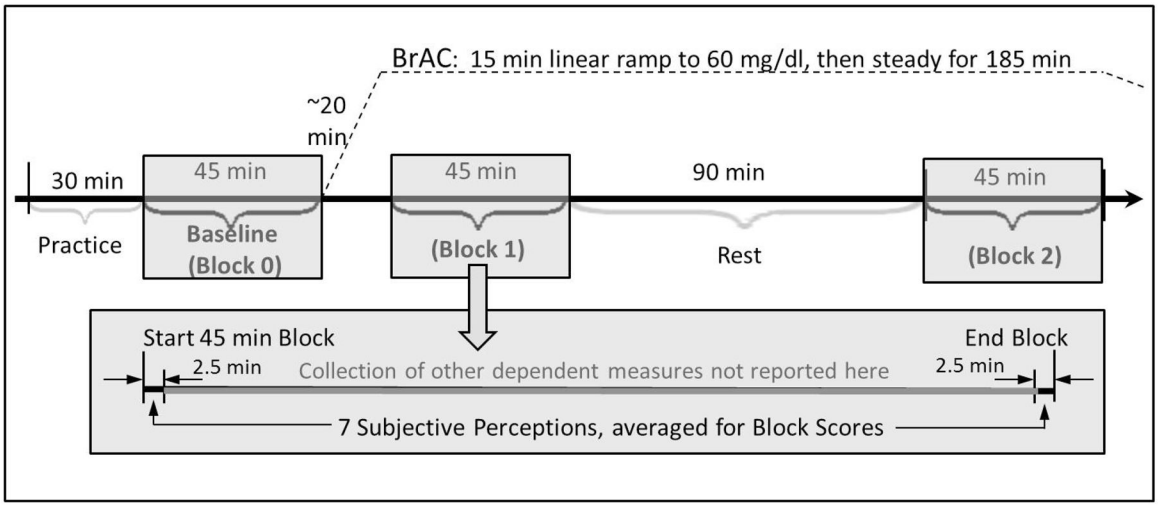

Figure 1. Session design

Subjective perceptions of alcohol were collected at the beginning and end of Block 0, Block 1, and Block 2, then averaged to give a single measurement for each block.

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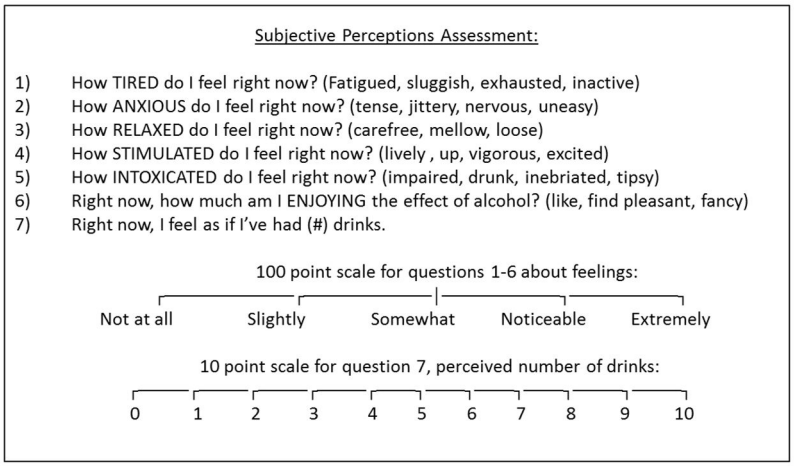

Figure 2. The Subjective Perceptions Assessment

The 7 questions were presented one at a time, in random order, at the beginning and end of each block. For questions 1–6, subjects rated their perceptions by moving a pointer along a visual analog scale anchored at 5 positions (coded from 0–100). For question 7, subjects estimated how many drinks it would take to create their current perceptions by moving a pointer on a scale ranging from 0 to 10 in half-drink increments. After the first assessment, the pointer was presented in the position selected for that question on the previous assessment. During the practice session, subjects were instructed to place the slider at 0 for number of Drinks, and at "Not at all" for Intoxicated and Enjoy. For Stimulated, Tired, Relaxed and Anxious, they were told to move the slider to reflect their current perceptions, independent of the effect of alcohol. Subjects were required to read each question aloud before moving the slider. Responses were automatically time-stamped and logged; subjects were able to answer all 7 questions in less than a minute.

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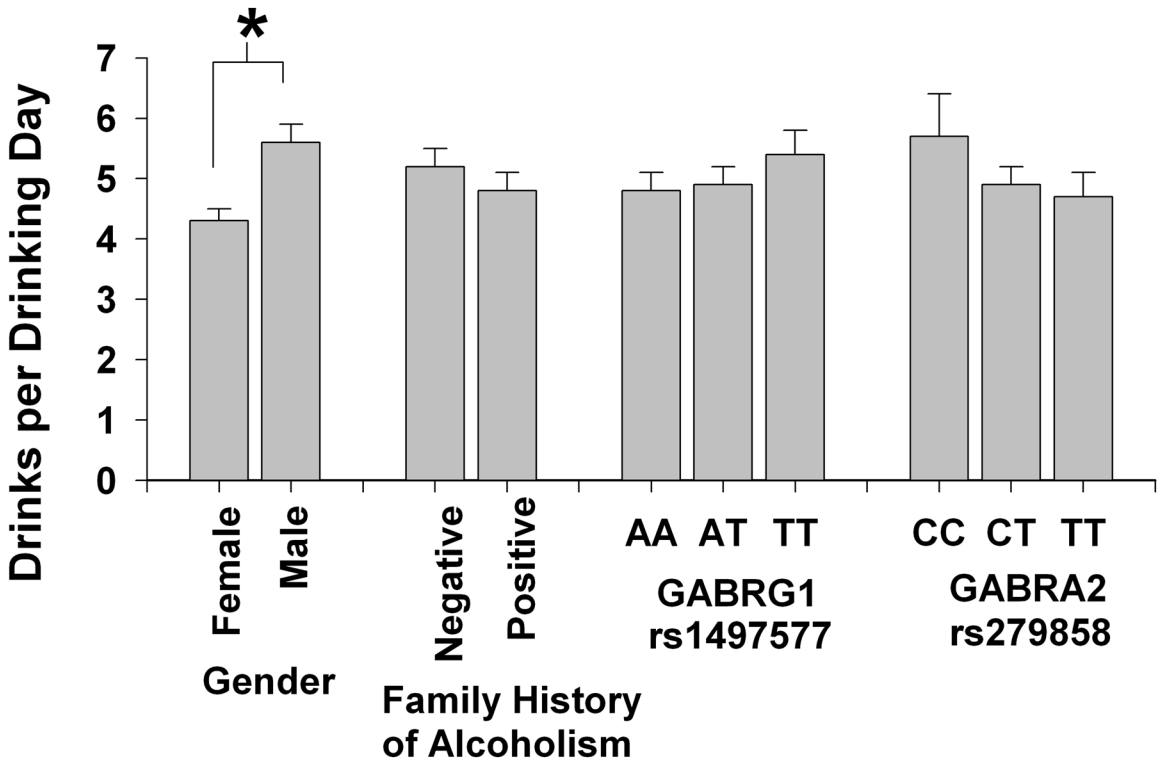

Figure 3. Recent drinking history by subgroup

Mean and SEM for recent drinking history (drinks/drinking day in the last 30 days) grouped by gender, family history of alcoholism, *GABRG1* genotype, and *GABRA2* genotype. * indicates P=0.001.

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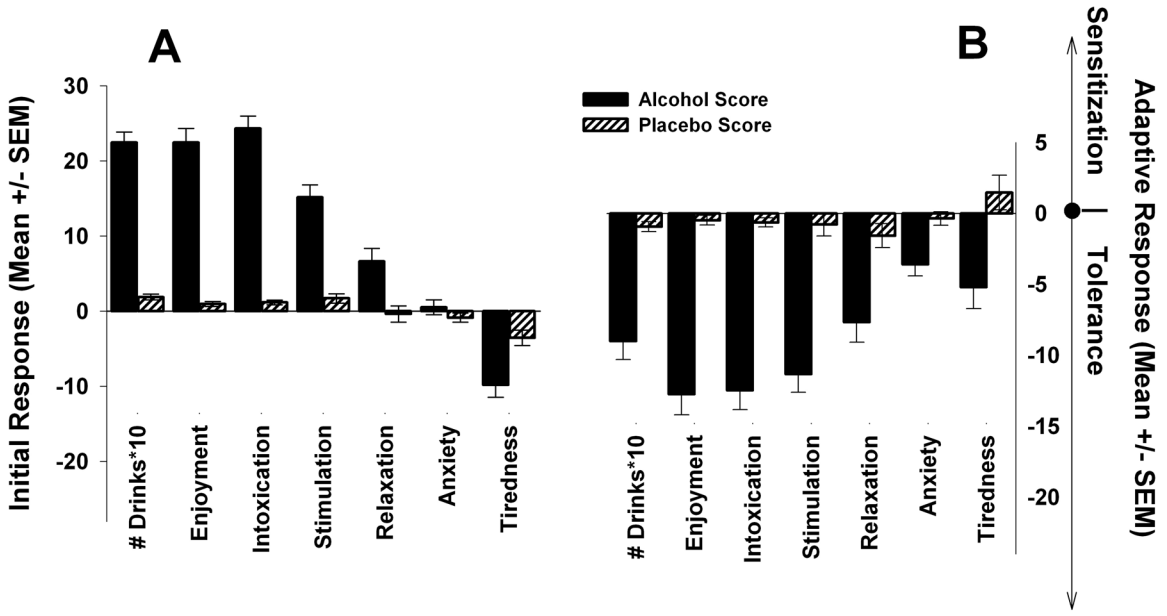

Figure 4. Initial and Adaptive Response to Alcohol

Mean and SEM of (A.) initial and (B.) adaptive response to alcohol scores for each of the seven subjective measures are shown. Black bars represent the mean scores for all subjects during the alcohol infusion; striped bars represent the mean scores during the placebo (saline) infusion; estimated number of drinks was multiplied by 10 so that it could be shown on the same scale as the other measures. Negative values of the adaptive response to alcohol reflect acute (within-session) tolerance (return toward baseline), while positive values reflect acute sensitization.

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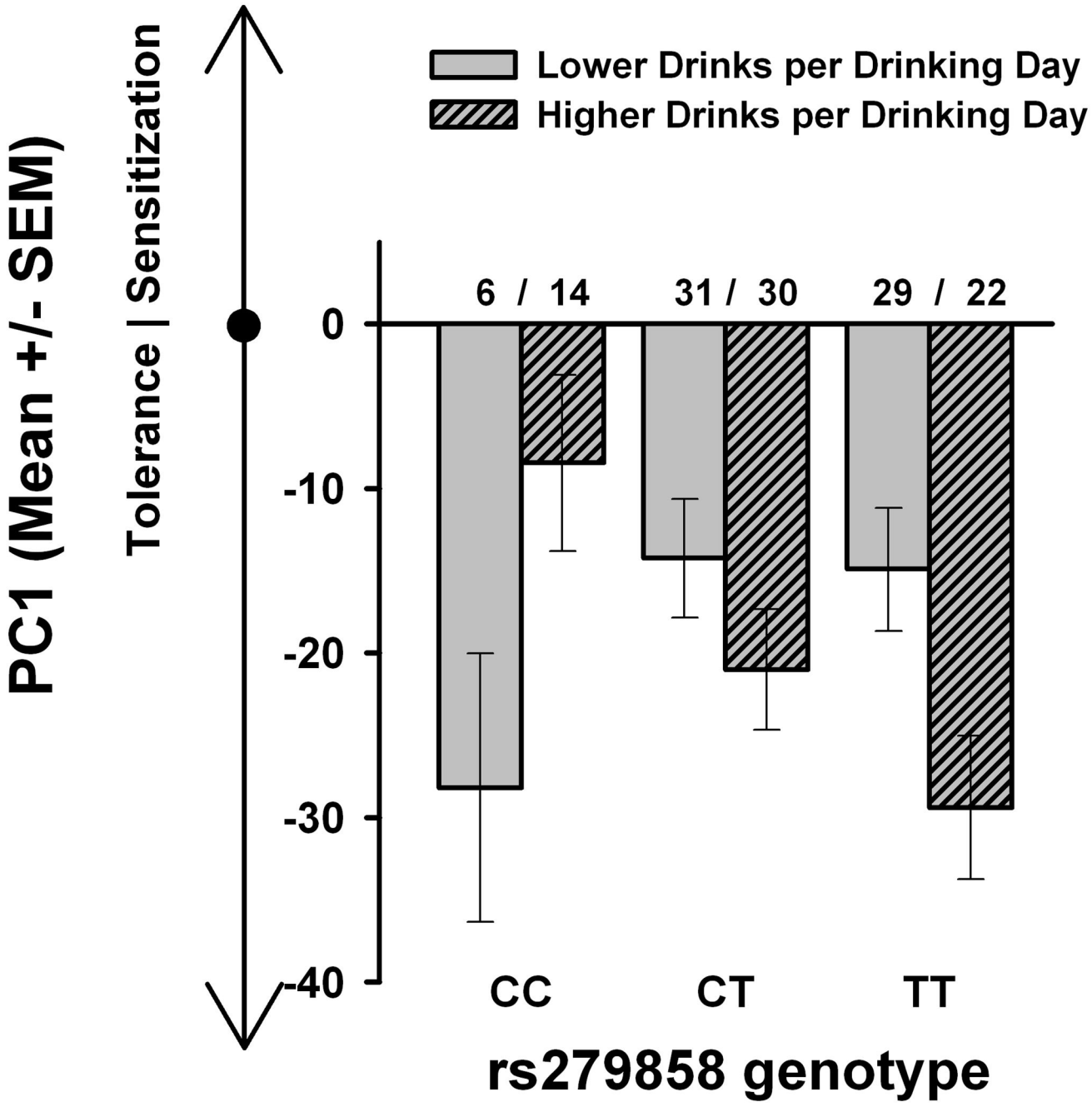

Figure 5. Adaptive response to alcohol as moderated by genotype and recent drinking history

This graph illustrates the significant genotype by recent drinking history interaction. Mean and SEM for PC1 is shown for *GABRA2* rs279858 genotypes, with subjects divided by a median split into either lower (black bars) or higher (striped bars) drinks per drinking day. Group N's are indicated above the bars. All groups, on average, showed tolerance, but the relative degree varied.

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Table 1

Subject numbers by subgroup and SNP information.

Subject numbers by subgroup	***GABRG1*: rs1497577 AA/AT/TT (n= 33/62/37)**	***GABRA2*: rs279858 CC/CT/TT (n= 20/61/51)**	**FHP/FHN (n=65/67)**
Females (n=63)	17/30/16	9/32/22	35/28
Males (n=69)	16/32/21	11/29/29	30/39
FHP (n=65)	15/30/20	11/27/27	
FHN (n=67)	18/32/17	9/34/24	
SNP information			
Position	46,093,713	46,314,593	
MAF	0.48	0.38	
Major (minor) allele	T(A)	T(C)	

FHP = family history positive for alcoholism, FHN = family history negative for alcoholism. Subject distributions did not differ for any groups (all $p \geq 0.17$).

Both SNPs were in Hardy-Weinberg equilibrium ($p > 0.53$), and demonstrated high heterozygosity (rs1497577 = 0.47; rs279858 = 0.46) and minor allele frequency (rs1497577 = 0.48; rs279858 = 0.38). MAF=Minor Allele Frequency;

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Table 2

Intercorrelations among subjective measures (Adaptive Response (placebo-adjusted))

	# of Drinks	Enjoyment	Intoxication	Stimulation	Relaxation	Anxiety	Tiredness
# of Drinks	1.00						
Enjoyment	**0.47**	1.00					
Intoxication	**0.76**	**0.59**	1.00				
Stimulation	0.21	**0.40**	0.33	1.00			
Relaxation	0.13	0.19	0.19	0.10	1.00		
Anxiety	0.01	0.02	0.02	0.22	0.12	1.00	
Tiredness	0.04	0.08	0.10	0.27	−0.07	0.09	1.00

Between variable correlation coefficients >0.40 are shown in boldface to highlight the higher correlations.

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Table 3

Principal Components for the adaptive response (Alcohol - Placebo): Weights, eigenvalues and % variability accounted for by the component.

	Subjective measure	PC1	PC2
Weights	Number of Drinks	**0.50**	−0.28
	Enjoyment	**0.50**	−0.08
	Intoxication	**0.55**	−0.20
	Stimulation	**0.37**	**0.47**
	Relaxation	0.20	−0.04
	Anxiety	0.09	**0.57**
	Tiredness	0.13	**0.57**
	Eigenvalue	**2.52**	**1.25**
	% variability	**36**	**18**

Weights >|0.3| are shown in boldface to indicate the measures that have a greater contribution to the component.

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Table 4

ANCOVA results for PC1, the aggregate measure of subjective perceptions.

ANCOVA results (F, df and p-values)							
Gene: SNP	**Overall F (df 5, 126)**	**Over all *p***	**FHA *p***	**Genotype *p***	**RDH *p***	**RDHx Genotype *p***	**Gender *p***
GABRG1: rs1497577	1.81	0.12	0.28	0.75	0.44	0.72	0.13
GABRA2: rs279858	2.82	**0.02**	0.10	0.10	**0.01**	**0.01**	0.10

Results for the analyses of the SNPs are shown in Table 4. Significant results are shown in boldface ($p<0.05$). FHA= Family History of Alcoholism; RDH = Recent Drinking History