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Effect of the TaqIA polymorphism on ethanol response in the brain

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Abstract

Acute ethanol administration increases striatal dopamine release and decreases cerebral glucose metabolism. The A1 allele of the ANKK1 TaqIA polymorphism is associated with lower dopaminergic tone and greater risk for alcoholism, but the mechanisms are unclear. We hypothesized that ethanol would be more reinforcing in men with the A1 allele (A1+) than in men without it (A1–), as indicated by decreased anxiety and fatigue and altered activity in associated brain regions. In a pilot study, A1+ and A1– men (6/group) drank ethanol (0.75 ml/kg) or placebo beverages on each of two days. Positron emission tomography with [F-18]fluorodeoxyglucose (FDG) was used to assess regional cerebral glucose metabolism as a measure of relative brain activity while participants performed a vigilance task. Ethanol decreased anxiety and fatigue in A1+ men but increased them in A1– men ($p = 0.008$). Ethanol increased activity in the striatum ($p = 0.005$) and insula ($p = 0.001$) of A1+ men, but reduced activity in the anterior cingulate of A1– men ($p = 0.03$). Reduced anxiety and fatigue in A1+ men were significantly associated with greater activity within a right orbitofrontal region previously implicated in cognitive control, and less activity in structures associated with anxiety (amygdala), fatigue (thalamus), and craving/reinforcement (striatum). In contrast, anxiety and fatigue changes were unrelated to brain activity in A1– men. Although these results require replication in a larger sample, alcohol-induced negative reinforcement may explain the greater risk for alcoholism associated with the A1 allele.

Keywords

Alcohol genetics; PET; addiction vulnerability; cerebral glucose metabolism; dopamine; negative reinforcement

1. Introduction

A growing literature points to an important role of genotype in vulnerability to substance abuse, with much of this work centering on the role of central dopamine systems in motivation and reinforcement (Volkow et al. 2007). Reduced density of striatal D2 dopamine receptors has been associated with reinforcer-induced impulsivity in rats (Dalley et al. 2007), and with presence of the TaqIA A1 allele of the ANKK1 gene (previously reported as located in the D2 dopamine receptor gene) (Neville et al. 2004) in humans, both through *in vitro* (Noble et al. 1991; Thompson et al. 1997) and *in vivo* (Jonsson et al. 1999; Pohjalainen et al. 1998) studies. Although A1 prevalence differs between ethnic groups (Barr and Kidd 1993), raising the possibility of stratification bias, studies both across and within diverse ethnic groups have reported robust associations of the polymorphism first with misuse of alcohol (Blum et al. 1990) and subsequently with other forms of substance abuse (Li et al. 2004a; Noble 2003; Young et al. 2004). In a meta-analysis of 3,329 Caucasian adults, the prevalence of the A1 allele (A1A1 and A1A2 genotypes) was significantly higher ($p = 1.54 \times 10^{-8}$) in alcoholics (38.9%) than nonalcoholics (29.4%) (Noble 2003). Effect sizes are often small, however, (Berggren et al. 2006), requiring large samples or meta-analyses to show associations in the absence of more strongly linked endophenotypes (White et al. 2008).

These associations have led to the hypothesis that the lower dopaminergic tone of the mesocorticolimbic reinforcement circuits in individuals with the A1 allele produces reinforcement-deficiencies or anhedonia (Blum et al. 2000; Noble 2000). Low D2 function is thought to 1) increase the reward value of direct D2 agonists, including all drugs of abuse, 2) decrease the reward value of less potent natural reinforcers, and 3) decrease the capacity for frontocortical inhibition, all of which contribute to compulsive drug taking (Volkow et al. 2004).

A report consistent with this view indicated that individuals with the A1 allele are characterized by less activation of the mesocorticolimbic reinforcement system than those without the allele (Cohen et al. 2005). In contrast, treatment with the D2 agonist bromocriptine enhanced activation of the reward system in individuals with, but not without the A1 allele (Kirsch et al. 2006).

We have previously shown that presence of the A1 allele in adolescents is associated with abnormal levels of, or abnormal relationships between, constructs related to hedonic tone, including family stress (Berman and Noble 1997), the personality characteristics of novelty seeking and harm avoidance (Berman et al. 2002), and negative affect (Berman et al. 2003). In PTSD-afflicted veterans, we found greater anxiety/insomnia, social dysfunction and depression in those with than without the A1 allele (Lawford et al. 2006). In combination with a gene coding for reduced dopamine transporter density, presence of the A1 allele has recently been associated both with higher levels of anxiety (Kulikova et al. 2008b), and mental fatigue (Kulikova et al. 2008a), as compared with individuals who did not combine these genotypes. In sum, the studies to date suggest that individuals with the A1 allele may be more susceptible to stress-induced anxiety and other negative affective states, and that these individuals are more likely to self-medicate negative affective states with dopamine-releasing drugs, such as alcohol.

The role of the A1 allele in mediating acute effects of alcohol is unknown. Alcohol consumption increases arousal and reduces anxiety in some individuals, while others primarily report increased fatigue. It increases striatal dopamine release, which has been associated with drug-mediated reward (Koob and Le Moal 2008; Yoder et al. 2008), and decreases cerebral glucose metabolism (de Wit et al. 1990; Volkow et al. 2006b). To test the hypothesis that alcohol is more reinforcing in men with the A1 allele, we assessed self-reported anxiety and fatigue and cerebral metabolic responses to acute ethanol administration. We hypothesized that in men

with the A1 allele ethanol would reduce anxiety, fatigue, and activity in brain structures associated with these unpleasant states more than in men without it.

2. Methods

2.1. Study design

Effects of ethanol were compared between social drinkers with no copies of the A1 allele (A1 – group), and those with one or two copies (A1+ group). Cerebral glucose metabolism was assessed using positron emission tomography (PET) while participants were engaged in an auditory continuous performance test (CPT) in two sessions, counterbalanced for order, 1–21 days apart (mean $\pm$ SD = 10.4 $\pm$ 7.8 days). In one session, participants drank a 250-ml mixture of caffeine-free diet soda plus a moderate dose of ethanol (0.75 g/kg body weight) equivalent to 1.4 ml of ethanol/ml of body water (Savoie et al. 1988)). In the other session, a non-pharmacologically relevant amount of ethanol was floated on top of the soda to simulate the taste and smell of a mixed beverage. In each session, anxiety and fatigue were self-reported. [F-18]Fluorodeoxyglucose (FDG) was administered to assess cerebral glucose metabolism, a measure of regional brain function (Phelps et al. 1979; Reivich et al. 1979), and raw counts from FDG, scaled to the global mean, were used as a surrogate index of regional cerebral glucose metabolism (rCMRglc). Relative activity, as used in this report, refers to this measure. Blood alcohol content was assessed via breathalyzer at least once per hour until recording a reading of 0.02% or lower. If all other study procedures were completed, the subject was then released.

2.2. Research participants

Participants were recruited through advertisements in local newspapers. Volunteers who met initial criteria in a telephone screening came to UCLA. After giving written informed consent, as approved by the UCLA Office for Protection of Research Subjects, they underwent a comprehensive evaluation, including medical history, physical examination, psychiatric interview (Structured Clinical Inventory for DSM-IV (SCID-I) (First et al. 1996), and completion of the Beck Depression Inventory (Beck et al. 1961), the Wender Utah Rating Scale for attention-deficit hyperactivity disorder (ADHD) (Ward et al. 1993), and a drug use survey.

Symptoms of depression (scores >18 on the Beck Depression Inventory) or ADHD (scores ≥ 46 on the Wender Utah Rating Scale) were exclusionary, as were history of neurological disease, head trauma with loss of consciousness > 5 min, claustrophobia, systemic disease, HIV-seropositive status, lifetime history of any DSM-IV Axis I or II psychiatric diagnosis, and history of dependence on alcohol or an illicit drug of abuse. Habitual use of caffeine or nicotine and light use of marijuana (≤ 1 cigarette per week) or alcohol (<90 g absolute alcohol per week) were allowed. Twelve right-handed (score > 20 on a modified version of the Edinburgh Handedness Test (Oldfield 1971)) Caucasian men aged 21 to 39 (mean [sd] = 29.0 [5.2]) completed the study and were paid for their participation.

2.3. Genotype analysis

A 10-ml blood sample was drawn from each subject. Genomic DNA was extracted and used as a template for determination of TaqI A alleles by the polymerase chain reaction (Grandy et al. 1993). The amplification of DNA was carried out using a Perkin Elmer GeneAmp 9600 Thermocycler. Approximately 500 ng of amplified DNA was digested with five units of TaqI restriction enzyme (New England Biolabs) at 65°C overnight. The resulting products were separated by electrophoresis in a 2.5% agarose gel containing ethidium bromide and visualized under ultraviolet light. Three genotypes are revealed: A1A2 (three fragments: 310, 180 and 130bp), A2A2 (two fragments: 180 and 130 bp), and A1A1 (one uncleaved 310 bp fragment).

A1+ allele subjects had either the A1A1 or A1A2 genotype; A1– allele subjects had the A2A2 genotype only.

2.4. Experimental sessions

Participants abstained from alcohol and marijuana for 48 h before each test session but used coffee and/or tobacco as usual. At each session, recent drug and alcohol use were evaluated by a urine drug screen and breathalyzer test. All participants received a standard non-ketogenic breakfast (egg, juice, toast) upon arriving at UCLA. After breakfast, a venous catheter was inserted for infusing FDG. PET images were acquired with a Siemens ECAT EXACT HR+ tomograph (CTI, Knoxville, TN) in 3D mode. A plastic facemask (Strymon Systems, Annapolis, MD) was fitted to each subject to minimize head motion. A 3-min ^{68}Ge transmission scan verified the position of the brain, and a 20-min ^{68}Ge transmission scan was performed for attenuation correction.

Participants were removed from the gantry, and trained on the CPT, which requires a button press prompted by a tone of designated pitch within a sequence of nontarget tones (inter-stimulus interval = 2 sec). This task was used to standardize the cognitive set during assessment of rCMRglc. After training, participants drank the experimental beverage over 5 min and then provided self-ratings of the tension-anxiety and fatigue-inertia subscales from the Profile of Mood States (McNair et al. 1971). The 30-min CPT was then initiated. Five min later, FDG ($\leq 5 \text{ mCi}$, $\leq 185 \text{ MBq}$) was injected. When the CPT ended, the subject was repositioned in the scanner gantry, and brain images were acquired for 30 min. Since the FDG was injected within the first half hour after alcohol consumption, and peak alcohol concentration is attained in 40–50 min (Sammi et al. 2000), uptake of the radiotracer presumably occurred during the ascending limb of the blood alcohol curve.

We reconstructed 128×128 pixel images using a Hann filter (cut-off frequency = 0.5 cycles/pixel). The average transverse resolutions at 1 and 10 cm from the center of the field-of-view (FOV), measured in 3D mode and determined using a ^{18}F line source, were 6.52 and 7.16 mm (full-width at half maximum, FWHM), and the average axial resolutions (FWHM) were 3.72 mm and 5.64 mm at 0 cm and 10 cm from the center of the FOV, respectively.

2.5. Data analysis

Response accuracy (% Hits, % False Alarms) and reaction time (RT) quantified vigilance performance at 15 and 30 min. Two-tailed t-tests compared the groups on vigilance and demographic variables. Chi square analyses were used for frequency data. The statistical threshold was set at $p < 0.05$.

PET images were converted into ANALYZE[®] format for analysis using statistical parametric mapping software (SPM5, <http://www.fil.ion.ucl.ac.uk/spm>). Each image was normalized into the standard coordinate system developed at the Montreal Neurological Institute (MNI space) by linear and non-linear transformation to a template image and smoothed with an 8-mm isotropic Gaussian kernel (voxel size $2 \times 2 \times 2 \text{ mm}^3$). Effects of global activity were removed by proportional scaling.

The images were analyzed on a voxel-by-voxel basis according to the general linear model by calculating statistical parametric maps (Friston et al. 1995). A parametric statistical model was assumed at each voxel to describe the variability in terms of experimental effects, and residual variability. Directional differences in group means were assessed at each voxel with Student's *t*-tests, producing images with voxel values equalling the *t* statistics. The multiple comparisons problem was addressed by modeling the image as a sample of a continuous Gaussian random field. For each voxel, the corrected voxel *p*-value is the probability under the null hypothesis

of finding at least one voxel with an equal or larger t within the search volume. For each cluster of contiguous voxels with suprathreshold t -values, the cluster (spatial extent) p -value is the probability of finding at least one cluster that large or larger within the search volume.

The effect of ethanol on relative activity was assessed by comparing the PET scans during the ethanol session with the corresponding scans during the placebo session, using a 2-group (A1+, A1-), 2-session (ethanol, placebo) design. The interaction of session and group quantified differences between the allelic groups in the effects of ethanol. To interpret interactions, planned contrasts assessed ethanol effects separately within each group. Covariate analyses quantified between-session correlations between change in anxiety and fatigue and change in relative brain activity, and group differences in the slope of this relationship.

We selected six regions of interest (ROIs) in each hemisphere on the basis of previous association with alcohol-mediated change in affect or reinforcement. The thalamus has been linked with alcohol-mediated fatigue and sedation (Jia et al. 2007; Volkow et al. 2006b), the amygdala with anxiety (Koob and Le Moal 2008; Silberman et al. 2008), the insula (BA 13) with interoceptive mood or “gut-feelings” (Craig 2002; Ingvar et al. 1998), the dorsal and ventral striatum with craving, reward and intoxication (Koob and Le Moal 2008; Yoder et al. 2008), and the rostral anterior cingulate cortex (BA 24, 32, 33) with executive-modulation of alcohol feelings (Ingvar et al. 1998; Sinha and Li 2007).

ROIs were drawn within each cerebral hemisphere on a structural MR template, using MEDx (Sensor Systems, Sterling, VA, USA), and aided by the use of a standardized stereotaxic atlas of the human brain (Talairach and Tournoux 1988). Whole-brain analyses provided exploratory data for other brain structures. The voxel height threshold for inclusion in clusters was $t = 3.22$, ($p = 0.005$ uncorrected). We considered as evidential, however, only clusters that also retained a spatial extent p -value < 0.05 after correction for multiple comparisons within the whole-brain (> 400 voxels) or ROI search volume (required cluster size varied with the size of the ROI). Effects that attained a p value < 0.1 , using family-wise error correction, were considered as suggestive trends. ROI results which retained statistical significance after Bonferroni-correction for the total number of one-tailed comparisons (i.e., 2 comparisons [A1+ > A1-, A1- > A1+] $\times$ 12 ROIs = 24; $0.05 / 24 = 0.0021$), were also identified.

3. Results

3.1. Blood alcohol content (BAC)

All placebo sessions generated breathalyzer values of 0.00% before and after beverage consumption. All alcohol sessions generated values of 0.00% before, and peak values that did not significantly differ between allelic groups after beverage consumption (0.03 – 0.07 in A1-; 0.03 – 0.11 in A1+). The precise peak values and timecourse of the BAC could not be calculated due to sparse temporal sampling, but it has been recently reported that consumption of two to three drinks produces BACs of 0.05– 0.06 % in 5–6 min (Biller et al. 2009; Heidelberg_University_Hospital 2009).

3.2. Vigilance task performance

The participants performed the vigilance task well after drinking both the placebo beverage (% Hits [mean $\pm$ SD] 98.5 ± 3.1 ; % False Alarms [FA] 1.1 ± 3.5 ; Reaction time [RT] 899 ± 298 msec) and ethanol (% Hits 98.8 ± 2.4 ; % FA 0.8 ± 2.4 ; RT 938 ± 344 msec). RT increased from 624 msec at 15 min to 919 msec at the final 30 min assessment ($F_{1,10} = 87.46$, $p < 0.001$), suggesting that vigilance decreased with time. There were no significant effects of beverage, allelic group, or their interaction on task performance.

3.3. Ethanol effects on rCMRglc

The effects of ethanol as compared to placebo on brain activity in the regions of interest are given in Table 1. There was evidence that ethanol increased relative rCMRglc in the dorsal striatum and amygdala across groups, and that presence of the A1 allele altered the effect of ethanol on rCMRglc (corrected Group $\times$ Condition interaction) in the insula and anterior cingulate (Figure 1). There also was a trend for an interaction in the right ventral striatum (spatial extent $p = 0.08$; 8, 12, -14 voxel $p = 0.08$). Planned comparisons within each group indicated that ethanol produced a relative increase in activity within the insula and striatum only in the A1+ group, and a relative decrease in anterior cingulate activity only in the A1–group. The statistical significance of the A1+ effect in the right insula was maintained after Bonferroni correction for the number of ROIs.

3.4. Anxiety and fatigue

Self-ratings of anxiety and fatigue during the placebo session were subtracted from those obtained during the alcohol session. The two measures were summed to yield a composite Anxiety/Fatigue score (see Figure 2A). When ethanol ingestion was associated with less anxiety and fatigue than placebo ingestion, the Alcohol minus Placebo subtraction score had a negative value. One-tailed t-tests of group differences in anxiety (A1–: [mean $\pm$ SD] 0.67 ± 1.75 ; A1+: -1.50 ± 2.43 ; $p = 0.05$), fatigue (A1–: 1.83 ± 2.23 ; A1+: -0.67 ± 2.50 ; $p = 0.05$) and the composite of the two measures (A1–: 2.50 ± 2.07 ; A1+: -2.17 ± 3.37 ; $p = 0.008$) suggested that ethanol increases anxiety/fatigue in the A1– allelic group, but decreases anxiety/fatigue in the A1+ allelic group.

3.5. Covariation between ethanol effects on anxiety/fatigue and rCMRglc

The relationship of ethanol-related change in anxiety/fatigue and rCMRglc were assessed through covariate analyses of the composite Alcohol-Placebo Anxiety/Fatigue score (see Table 2 and Figure 2B). Across all subjects, there was a positive correlation between Anxiety/Fatigue and activity in left anterior cingulate cortex and right thalamus, but a negative correlation with activity in left insula. Slope tests indicated that presence, as compared to absence of the A1 allele, was associated with more positive slope within the left insula and ventral striatum.

In planned comparisons within each group, the A1– group produced no evidence for positive correlations between activity and Anxiety/Fatigue (see lower portion of Table 2 and Figure 2B), and one negative correlation in the left insula. The interaction between allelic group and slope in the left dorsal insula reflected the negative correlation exhibited only in the A1– group.

In the A1+ group, an anteroventral insula cluster (15% of left insula) was the only negative correlation in an *a priori* ROI (230 voxels, spatial-extent $p = 0.005$; -42, 22, 2 voxel $t = 9.49$, $p = 0.003$). A significant positive correlation with activity in the A1+, but not A1– group produced the interaction in the left ventral striatum (Table 2). There were additional positive correlations in bilateral thalamus, amygdala, and striatum (Table 2). Effects retained significance after Bonferroni correction in the left amygdala (voxel criterion) and the right thalamus (spatial extent).

Although no *a priori* ROI exhibited significant findings for the A1–>A1+ interaction, a cluster of 405 voxels in right ventrolateral orbitofrontal cortex retained significance after correction for whole-brain volume (spatial extent $p < 0.05$, peak voxel $t = 8.79$, 46, 42, -8). Whole-brain corrected analyses indicated that this interaction represented negative correlation in the A1+ allelic group (798 voxels, spatial extent $p = 0.001$; 44, 38, -8 voxel $t = 15.52$, $p = 0.006$) with no positive correlation in the A1– group (both p 's > 0.9).

4. Discussion

Mood disorders constitute the greatest disease burden in the developed world. Alcohol use disorders are almost as costly, implicated in 20–40% of hospital admissions and frequently comorbid with mood disorders but having a later age of onset (Li et al. 2004b). The dominant psychosocial models developed to explain the relationship between anxiety disorders and alcohol use disorders posit that alcohol reduces anxiety and other uncomfortable responses to stress in predisposed individuals. This effect is thought to encourage chronic self-medication because of the temporary negative reinforcement alcohol can provide (Morris et al. 2005). Inconsistent empirical support for these models has underlined the importance of individual differences in risk for alcoholism (Sher and Levenson 1982), including gender, family history of alcoholism, and personality characteristics such as high novelty seeking and low harm avoidance (Croissant et al. 2008; Croissant et al. 2006).

Presence of the TaqIA A1 allele interacts with stress to predict cognitive functions in adolescents (Berman and Noble 1997) and alcoholism in adults (Madrid et al. 2001). It interacts with negative affect and gender to predict alcoholic personality characteristics (Berman et al. 2003) and severity of substance abuse in adolescents (Conner et al. 2005). Furthermore, among hospitalized substance abusers or alcoholics (Nixon and Parsons 1990), and drug-naïve adolescent boys carrying the A1 allele, but not among boys without it (Berman et al. 2002), the negative correlation between novelty seeking and harm avoidance that generally characterizes personality assessment is reversed. This finding suggests that rather than providing positive reinforcement, novelty seeking may serve a self-medicating negative reinforcement function in both severe substance abusers and in boys with the A1 allele. We predicted that acute ethanol administration would be more reinforcing in A1+ than A1– social drinkers, as indicated by ameliorated anxiety and fatigue, and altered brain activity in structures associated with negative affect.

Previous studies that used blood-sampling during PET to determine absolute metabolic rate for glucose have demonstrated that acute doses of ethanol equivalent to 2–3 mixed drinks produce robust decreases in cerebral glucose metabolism (> 20% in the posterior cerebral cortex) (Volkow et al. 2008; Volkow et al. 2006b). Figure 2 of the 2008 paper (Volkow et al. 2008), also presents relative (whole-brain normalized) metabolic images like those employed in the current study. These images depict a more modest relative decrease in posterior cortical metabolism and a relative increase in subcortical and temporal cortices, particularly in the left hemisphere. Although the current study formally assessed alcohol effects only within regions previously associated with affect or reinforcement, visual examination of results in posterior cortex confirmed the decreased relative metabolism previously reported (Volkow et al. 2008), just as the relative increases in striatal and amygdala metabolism in Table 1 are consistent with the prior results in these structures. These observations suggest that the relative increases in Table 1 and Figure 1 represent relative preservation of glucose metabolism (i.e., less decrease than the global average). Individual values in the areas of interest are consistent with A1+ men but not A1– men having relatively preserved glucose metabolism in the insula and striatum, and with A1– men having a greater than whole brain decrease in glucose metabolism in the anterior cingulate cortex.

Although dopamine release in the nucleus accumbens has been associated with alcohol-related reinforcement (Volkow et al. 2007), dopamine release in the dorsal striatum, where the current effects are largest, has also been associated with craving for alcohol (Heinz et al. 2005), and for other drugs (Volkow et al. 2006c; Wong et al. 2006). We interpret relative preservation of activity in the striatum and amygdala as indicating affective responses to ethanol, and the greater preservation of activity in the striatum and insula in A1+ men as suggesting larger affective responses. In A1– men, reduction of activity by ethanol in the rostral anterior

cingulate, an area associated with cognitive modulation of alcohol feelings (Ingvar et al. 1998; Sinha and Li 2007), may indicate fewer alcohol-related feelings, or less ability to modify them.

The lower anxiety and fatigue after ethanol in A1+ men, compared with the higher anxiety and fatigue in A1– men, strongly supports the hypothesis that ethanol is more reinforcing in A1 carriers. The increased negative affect in A1– men is consistent with a recent fMRI study where the dopamine agonist bromocriptine improved speeded motor performance and activation of the nucleus accumbens in A1 carriers, who performed worse than noncarriers under placebo and improved less in a high-incentive condition, but whose deficits were abolished by bromocriptine (Kirsch et al. 2006). In contrast, bromocriptine lowered performance in A1– individuals, just as ethanol worsened negative affect in our A1– men. The authors suggested that an inverted ‘U’ function may characterize the relationship of dopamine activity to optimal functioning, and that A1 carriers are characterized by both a reward deficiency syndrome and increased delivery of reward by dopamine agonists.

The latter idea is further supported by the current relationship between ethanol-induced change in negative affect and regional brain activity (Table 2). Positive covariation of anxiety/fatigue score with thalamic activity across groups is consistent with previous studies of alcohol-induced fatigue (Jia et al. 2007; Volkow et al. 2006b). Positive covariation of these scores with ACC activity is consistent with executive-modulation of alcohol feelings (Ingvar et al. 1998; Sinha and Li 2007). Negative covariation of concurrent changes in anxiety/fatigue and activity in a cluster comprising 15% of the left insula in A1+ men suggests that ethanol-related reduction in anxiety/fatigue (see Figure 2) may reflect the positive interoceptive feelings produced by alcohol, sometimes referred to as a “warm glow.” In A1– men, the more dorsal negative covariation in 7% of left insula is harder to interpret and may be spurious, since it is the only one of 24 comparisons to produce a $p < 0.05$ cluster (see Table 2).

The dearth of relationships between the effects of ethanol on negative affect and brain activity in A1– men is, however, consistent with a report that nonalcoholic members of alcoholic families exhibit higher D2 receptor availability than alcoholics from the same families (Volkow et al. 2006a). The study also found associations between D2 availability and glucose metabolism in frontal regions implicated in emotional reactivity and executive control. We speculate that higher levels of D2 receptors in A1– men (Jonsson et al. 1999; Noble 2003) could protect against alcoholism by improving regulation of emotional responses that might otherwise lead to drug-seeking.

In contrast to the lack of evidence that changed negative affect was related to change in brain activity in A1– men, the decreased anxiety and fatigue produced by ethanol in A1+ men accompanied decreased activity in the structures most consistently associated with both anxiety (amygdala) (Koob and Le Moal 2008; Silberman et al. 2008), and fatigue (thalamus) (Jia et al. 2007; Volkow et al. 2006b). Both effects survived a conservative Bonferroni correction. Table 2 also shows decreases in three of four ROIs comprising a structure previously associated with craving (striatum) (Koob and Le Moal 2008; Yoder et al. 2008). In addition, reduced negative affect after ethanol in the A1+ group was accompanied by significant activation of right ventrolateral orbitofrontal cortex. Activity in this region has been associated with lower stress, inhibition of stress-related amygdala activity, and higher psychosocial resources for threat-regulation (Taylor et al. 2008).

Limitations of this pilot study include a lack of detailed information on lifetime alcohol consumption, and the restriction of testing to Caucasian males. Although these findings require replication in larger and more diverse samples, they provide the first evidence that the

heightened risk for alcoholism associated with the DRD2 A1 allele may be mediated by increased reward sensitivity and negative reinforcement when drinking alcohol.

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References

- Barr CL, Kidd KK. Population frequencies of the A1 allele at the dopamine D2 receptor locus. *Biol Psychiatry* 1993;34:204–209. [PubMed: 8399816]
- Beck AT, Ward CH, Mednelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Archives of General Psychiatry* 1961;4:561–571. [PubMed: 13688369]
- Berggren U, Fahlke C, Aronsson E, Karanti A, Eriksson M, Blennow K, Thelle D, Zetterberg H, Balldin J. The taqI DRD2 A1 allele is associated with alcohol-dependence although its effect size is small. *Alcohol and Alcoholism* 2006;41:479–485. [PubMed: 16751215]
- Berman S, Ozkaragoz T, Young RM, Noble EP. D2 dopamine receptor gene polymorphism discriminates two kinds of novelty seeking. *Personality and Individual Differences* 2002;33:867–882.
- Berman SM, Noble EP. The D 2 dopamine receptor (DRD2) gene and family stress; interactive effects on cognitive functions in children. *Behavior Genetics* 1997;27:33–43. [PubMed: 9145542]
- Berman SM, Ozkaragoz T, Noble EP, Antolin T, Sheen C, Siddarth P, Conner BT, Ritchie T. Differential associations of sex and D2 dopamine receptor (DRD2) genotype with negative affect and other substance abuse risk markers in children of alcoholics. *Alcohol* 2003;30:201–210. [PubMed: 13679114]
- Billar A, Bartsch AJ, Homola G, Solymosi L, Bendszus M. The effect of ethanol on human brain metabolites longitudinally characterized by proton MR spectroscopy. *J Cereb Blood Flow Metab* 2009;29:891–902. [PubMed: 19240741]
- Blum K, Braverman ER, Holder JM, Lubar JF, Monastra VJ, Miller D, Lubar JO, Chen TJ, Comings DE. Reward deficiency syndrome: a biogenetic model for the diagnosis and treatment of impulsive, addictive, and compulsive behaviors. *Journal of Psychoactive Drugs* 2000;32:1–112.
- Blum K, Noble EP, Sheridan PJ, Montgomery A, Ritchie T, Jagadeeswaran P, Nogami H, Briggs AH, Cohn JB. Allelic association of human dopamine D 2 receptor gene in alcoholism. *Journal of the American Medical Association* 1990;263:2055–2260. [PubMed: 1969501]
- Cohen MX, Young J, Baek JM, Kessler C, Ranganath C. Individual differences in extraversion and dopamine genetics predict neural reward responses. *Cognitive Brain Research* 2005;25:851–861.
- Conner BT, Noble EP, Berman SM, Ozkaragoz T, Ritchie T, Antolin T, Sheen C. DRD2 genotypes and substance use in adolescent children of alcoholics. *Drug and Alcohol Dependence* 2005;79:379–387. [PubMed: 16102380]
- Craig AD. How do you feel? Interoception: the sense of the physiological condition of the body. *Nature Reviews Neuroscience* 2002;3:655–666.
- Croissant B, Demmel R, Rist F, Olbrich R. Exploring the link between gender, sensation seeking, and family history of alcoholism in cortisol stress-response dampening. *Biological Psychology* 2008;79:268–274. [PubMed: 18675310]
- Croissant B, Rist F, Demmel R, Olbrich R. Alcohol-induced heart rate response dampening during aversive and rewarding stress paradigms in subjects at risk for alcoholism. *International Journal of Psychophysiology* 2006;61:253–261. [PubMed: 16386812]
- Dalley JW, Fryer TD, Brichard L, Robinson ES, Theobald DE, Laane K, Pena Y, Murphy ER, Shah Y, Probst K, Abakumova I, Aigbirhio FI, Richards HK, Hong Y, Baron JC, Everitt BJ, Robbins TW. Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. *Science* 2007;315:1267–1270. [PubMed: 17332411]
- de Wit H, Metz J, Wagner N, Cooper M. Behavioral and subjective effects of ethanol: Relationship to cerebral metabolism using PET. *Alcoholism, Clinical and Experimental Research* 1990;14:482–489.

- First, MB.; Spitzer, RL.; Gibbon, M.; Williams, J. Biometrics Research Department. New York, NY: New York State Psychiatric Institute; 1996. Structured Clinical Interview for DSM-IV Axis I Disorders- Patient Edition (SCID-IP, Version 2.0).
- Friston KJ, Holmes AP, Worsley KJ, Poline JP, Frith CD, Frackowiak RSJ. Statistical parametric maps in functional imaging: A general linear approach. *Human Brain Mapping* 1995;2:189–210.
- Grandy DK, Zhang Y, Civelli O. PCR detection of the TaqA RFLP at the DRD2 locus. *Human Molecular Genetics* 1993;2:2197. [PubMed: 7906592]
- Heidelberg_University_Hospital. From The Glass To The Brain In Six Minutes. *Science Daily*. 2009 Jun 15. <http://www.sciencedaily.com/releases/2009/06/090615144339.htm>
- Heinz A, Siessmeier T, Wrase J, Buchholz HG, Grunder G, Kumakura Y, Cumming P, Schreckenberger M, Smolka MN, Rosch F, Mann K, Bartenstein P. Correlation of alcohol craving with striatal dopamine synthesis capacity and D2/3 receptor availability: a combined [18F]DOPA and [18F]DMFP PET study in detoxified alcoholic patients. *American Journal of Psychiatry* 2005;162:1515–1520. [PubMed: 16055774]
- Ingvar M, Ghatan PH, Wirsén-Meurling A, Risberg J, Von Heijne G, Stone-Elander S, Ingvar DH. Alcohol activates the cerebral reward system in man. *Journal of Studies on Alcohol* 1998;59:258–269. [PubMed: 9598706]
- Jia F, Pignataro L, Harrison NL. GABAA receptors in the thalamus: alpha4 subunit expression and alcohol sensitivity. *Alcohol* 2007;41:177–185. [PubMed: 17521848]
- Jonsson EG, Nothen MM, Grunhage F, Farde L, Nakashima Y, Propping P, Sedvall GC. Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. *Molecular Psychiatry* 1999;4:290–296. [PubMed: 10395223]
- Kirsch P, Reuter M, Mier D, Lonsdorf T, Stark R, Gallhofer B, Vaitl D, Hennig J. Imaging gene-substance interactions: the effect of the DRD2 TaqIA polymorphism and the dopamine agonist bromocriptine on the brain activation during the anticipation of reward. *Neurosci Lett* 2006;405:196–201. [PubMed: 16901644]
- Koob GF, Le Moal M. Addiction and the brain antireward system. *Annual Review of Psychology* 2008;59:29–53.
- Kulikova, M.; Timofeeva, M.; Maluchenko, N.; Tonevitsky, A. Effect of polymorphisms in the dopaminergic system genes on the mental fatigue. Presented at conference, “1st International ISBS Summer School on Behavioral Genetics and Neuroscience of Stress.”; St. Petersburg, Russia. 2008a.
- Kulikova MA, Trushkin EV, Timofeeva MA, Shlepzova VA, Schegolkova JV, Maluchenko NV, Tonevitsky AG. Genetic markers of predisposition to increased anxiety. *Bull Exp Biol Med* 2008b; 146:774–778. [PubMed: 19513381]
- Lawford BR, Young R, Noble EP, Kann B, Ritchie T. The D2 dopamine receptor (DRD2) gene is associated with co-morbid depression, anxiety and social dysfunction in untreated veterans with post-traumatic stress disorder. *Eur Psychiatry* 2006;21:180–185. [PubMed: 16632165]
- Li MD, Ma JZ, Beuten J. Progress in searching for susceptibility loci and genes for smoking-related behaviour. *Clinical Genetics* 2004a;66:382–392. [PubMed: 15479180]
- Li TK, Hewitt BG, Grant BF. Alcohol use disorders and mood disorders: a National Institute on Alcohol Abuse and Alcoholism perspective. *Biological Psychiatry* 2004b;56:718–720. [PubMed: 15556112]
- Madrid GA, MacMurray J, Lee JW, Anderson BA, Comings DE. Stress as a mediating factor in the association between the DRD2 TaqI polymorphism and alcoholism. *Alcohol* 2001;23:117–122. [PubMed: 11331109]
- McNair, D.; Lorr, M.; Droppleman, L. Profile of Mood States (Manual). San Diego: Educational and Industrial Testing Service; 1971.
- Morris EP, Stewart SH, Ham LS. The relationship between social anxiety disorder and alcohol use disorders: a critical review. *Clinical Psychology Review* 2005;25:734–760. [PubMed: 16042994]
- Neville MJ, Johnstone EC, Walton RT. Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. *Hum Mutat* 2004;23:540–545. [PubMed: 15146457]
- Nixon SJ, Parsons OA. Application of the Tridimensional Personality Questionnaire to a population of alcoholics and other substance abusers. *Alcoholism, clinical and experimental research* 1990;14:513–517.

- Noble EP. Addiction and its reward process through polymorphisms of the D 2 dopamine receptor gene: a review. *European Psychiatry* 2000;15:79–89. [PubMed: 10881203]
- Noble EP. D2 dopamine receptor gene in psychiatric and neurologic disorders and its phenotypes. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics)* 2003;116B:103–125.
- Noble EP, Blum K, Ritchie T, Montgomery A, Sheridan PJ. Allelic association of the D2 dopamine receptor gene with receptor-binding characteristics in alcoholism. *Archives of General Psychiatry* 1991;48:648–654. [PubMed: 2069496]
- Oldfield RC. The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia* 1971;9:97–113. [PubMed: 5146491]
- Phelps ME, Huang SC, Hoffman EJ, Selin C, Sokoloff L, Kuhl DE. Tomographic measurement of local cerebral glucose metabolic rate in humans with (F-18)2-fluoro-2-deoxy-D-glucose: Validation of method. *Annals of Neurology* 1979;6:371–388. [PubMed: 117743]
- Pohjalainen T, Rinne JO, Nagren K, Lehtikainen P, Anttila K, Syvalahti EK, Hietala J. The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. *Molecular Psychiatry* 1998;3:256–260. [PubMed: 9672901]
- Reivich M, Kuhl D, Wolf A, Greenberg J, Phelps M, Ido T, Casella V, Fowler J, Hoffman E, Alavi A, Som P, Sokoloff L. The [18 F]fluorodeoxyglucose method for the measurement of local cerebral glucose utilization in man. *Circulation Research* 1979;44:127–137. [PubMed: 363301]
- Sammi MK, Pan JW, Telang FW, Schuhlein D, Molina PE, Volkow ND, Springer CS, Hetherington HP. Measurements of human brain ethanol T(2) by spectroscopic imaging at 4 T. *Magn Reson Med* 2000;44:35–40. [PubMed: 10893519]
- Savoie TM, Emory EK, Moody-Thomas S. Acute alcohol intoxication in socially drinking female and male offspring of alcoholic fathers. *Journal of Studies on Alcohol* 1988;49:430–435. [PubMed: 3216646]
- Sher KJ, Levenson RW. Risk for alcoholism and individual differences in the stress-response-dampening effect of alcohol. *Journal of Abnormal Psychology* 1982;91:350–367. [PubMed: 7142573]
- Silberman Y, Shi L, Brunso-Bechtold JK, Weiner JL. Distinct mechanisms of ethanol potentiation of local and paracapsular GABAergic synapses in the rat basolateral amygdala. *Journal of pharmacology and experimental therapeutics* 2008;324:251–260. [PubMed: 17921186]
- Sinha R, Li CS. Imaging stress- and cue-induced drug and alcohol craving: association with relapse and clinical implications. *Drug and Alcohol Review* 2007;26:25–31. [PubMed: 17364833]
- Talairach, J.; Tournoux, P. Co-planar stereotaxic atlas of the human brain. New York, NY: Theme Medical Publishers Inc.; 1988.
- Taylor SE, Burklund LJ, Eisenberger NI, Lehman BJ, Hilmert CJ, Lieberman MD. Neural bases of moderation of cortisol stress responses by psychosocial resources. *Journal of personality and social psychology* 2008;95:197–211. [PubMed: 18605860]
- Thompson J, Thomas N, Singleton A, Piggott M, Lloyd S, Perry EK, Morris CM, Perry RH, Ferrier IN, Court JA. D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. *Pharmacogenetics* 1997;7:479–484. [PubMed: 9429233]
- Volkow ND, Fowler JS, Wang GJ, Swanson JM. Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. *Molecular Psychiatry* 2004;9:557–569. [PubMed: 15098002]
- Volkow ND, Fowler JS, Wang GJ, Swanson JM, Telang F. Dopamine in drug abuse and addiction: results of imaging studies and treatment implications. *Archives of Neurology* 2007;64:1575–1579. [PubMed: 17998440]
- Volkow ND, Ma Y, Zhu W, Fowler JS, Li J, Rao M, Mueller K, Pradhan K, Wong C, Wang GJ. Moderate doses of alcohol disrupt the functional organization of the human brain. *Psychiatry Research* 2008;162:205–213. [PubMed: 18280711]
- Volkow ND, Wang GJ, Begleiter H, Porjesz B, Fowler JS, Telang F, Wong C, Ma Y, Logan J, Goldstein R, Alexoff D, Thanos PK. High levels of dopamine D2 receptors in unaffected members of alcoholic families: possible protective factors. *Archives of General Psychiatry* 2006a;63:999–1008. [PubMed: 16953002]

- Volkow ND, Wang GJ, Franceschi D, Fowler JS, Thanos PP, Maynard L, Gatley SJ, Wong C, Veech RL, Kunos G, Kai Li T. Low doses of alcohol substantially decrease glucose metabolism in the human brain. *Neuroimage* 2006b;29:295–301. [PubMed: 16085426]
- Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Childress AR, Jayne M, Ma Y, Wong C. Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. *Journal of Neuroscience* 2006c;26:6583–6588. [PubMed: 16775146]
- Ward MF, Wender PH, Reimherr FW. The Wender Utah Rating Scale: An aid in the retrospective diagnosis of childhood attention deficit hyperactivity disorder. *American Journal of Psychiatry* 1993;150:885–890. [PubMed: 8494063]
- White MJ, Morris CP, Lawford BR, Young RM. Behavioral phenotypes of impulsivity related to the ANKK1 gene are independent of an acute stressor. *Behavior and Brain Function* 2008;4:54.
- Wong DF, Kuwabara H, Schretlen DJ, Bonson KR, Zhou Y, Nandi A, Brasic JR, Kimes AS, Maris MA, Kumar A, Contoreggi C, Links J, Ernst M, Rousset O, Zuckin S, Grace AA, Rohde C, Jasinski DR, Gjedde A, London ED. Increased occupancy of dopamine receptors in human striatum during cue-elicited cocaine craving. *Neuropsychopharmacology* 2006;31:2716–2727. [PubMed: 16971900]
- Yoder KK, Morris ED, Constantinescu CC, Cheng TE, Normandin MD, O'Connor SJ, Kareken DA. When What You See Isn't What You Get: Alcohol Cues, Alcohol Administration, Prediction Error, and Human Striatal Dopamine. *Alcoholism, clinical and experimental research*. 2008
- Young RM, Lawford BR, Nutting A, Noble EP. Advances in molecular genetics and the prevention and treatment of substance misuse: Implications of association studies of the A1 allele of the D2 dopamine receptor gene. *Addictive behaviors* 2004;29:1275–1294. [PubMed: 15345265]

Figure 1.

Effects of ethanol on relative regional glucose metabolism (rCMRglc). Data are from 12 men who were either A1+ or A1- ($n = 6$ for each group). Figures are depicted in neurological orientation. The gray-scale image is a T1 structural MRI that is representative of MNI space, where positive values of the x, y, and z coordinates approximately represent mm to the right, anterior and superior, relative to the midpoint of the anterior commissure. In pseudocolor brain images, values of $t > 3.22$ ($p < 0.005$) are indicated in the color bar. A. Colored voxels within the striatum (coronal slice) and amygdala (axial slice) indicate ethanol was associated with higher relative rCMRglc than placebo ($n = 12$).

B. Effects of ethanol ingestion on rCMRglc varied with presence of the A1 allele in the insula (left panel) and anterior cingulate cortex (right panel). Colored voxels on sagittal slices depict

greater relative rCMRglc after alcohol in the insula of A1+ men and less relative rCMRglc after alcohol in the anterior cingulate of A1- men. Mean cluster values for individual participants, plotted below as proportional change scores (Alc-Pla/ Pla), indicate the interactions in table 1 result from little or no overlap between the allelic groups. Bar height = mean +SD.

Figure 2. Effects of ethanol on anxiety and fatigue, and their relationship to rCMRglc, differed between groups. A. Self-ratings of anxiety and fatigue after placebo were subtracted from corresponding ratings after ethanol. A composite of both ratings differed between allelic groups ($p < 0.008$). Individual values are plotted to the right. Bar height = mean +SD; $n = 6$ per group. B. Colored voxels indicate that effects of ethanol on rCMRglc covaried with effects on composite anxiety + fatigue (all voxel $t > 3.22$, $p < 0.005$, volume-corrected spatial extent $p < 0.05$). In A1+ men, there was a positive correlation between ethanol's effects on anxiety/fatigue and metabolism in the amygdala, striatum & thalamus (red/yellow voxels), with negative correlations in the left insula and right ventrolateral prefrontal cortex (blue voxels). A1- men showed only a borderline negative correlation in the left insula.

Table 1

Effects of ethanol on relative regional glucose metabolism (rCMRglc).

Cluster-level					Voxel-level				
	Volume Corrected p Value	Cluster Size (voxels)	ROI Size	%ROI p<.005	Volume Corrected p Value	T	x	y	z
Main effects: Ethanol increased relative rCMRglc.									
Amygdala	0.034	15	144	10%	0.029	3.92	-22	0	-22
	-----	-----	-----	-----	-----	-----	-----	-----	-----
Dorsal striatum	0.079	50	993	6%	0.026	5.80	-10	16	4
	-----	-----	-----	-----	-----	-----	-----	-----	-----
Insula	0.023	121	1015	12%	0.033	5.66	12	12	12
Interactions: Ethanol increased relative rCMRglc in A1+ or decreased relative rCMRglc in A1-									
	-----	-----	-----	-----	-----	-----	-----	-----	-----
ACC	0.048	77	1446	5%	0.017	6.43	36	6	2
	-----	-----	-----	-----	-----	-----	-----	-----	-----
	0.046	66	1067	6%	0.040	5.20	-10	46	16
	-----	-----	-----	-----	-----	-----	-----	-----	-----
A1+ Group: Ethanol increased relative rCMRglc									
	-----	-----	-----	-----	-----	-----	-----	-----	-----
Insula	0.017	136	1446	9%	0.001**	9.93	36	6	2
	-----	-----	-----	-----	-----	-----	-----	-----	-----
Dorsal striatum	0.007	195	1015	21%	0.005	7.70	12	10	10
	-----	-----	-----	-----	-----	-----	-----	-----	-----
Ventral Striatum	0.038	18	184	10%	0.011	4.99	8	14	-14
A1-Group: Ethanol decreased relative rCMRglc									
	-----	-----	-----	-----	-----	-----	-----	-----	-----
ACC	0.039	75	1067	7%	0.030	5.51	-10	46	16
	-----	-----	-----	-----	-----	-----	-----	-----	-----

Results represent voxels within six regions of interest (ROIs: insula, amygdala, rostral anterior cingulate cortex [ACC], dorsal striatum, ventral striatum, thalamus) where effects of ethanol, as compared to placebo, passed a height threshold of $p < 0.005$, and where cluster size attained $p < 0.05$ for spatial extent after volume correction. Suggestive results are tabled for contralateral ROIs. All main effects represent higher relative rCMRglc after ethanol (i.e.- no clusters had significantly lower rCMRglc after ethanol). All interactions represent higher relative rCMRglc after ethanol in the A1+ group, and lower rCMRglc

after ethanol in the A1− group (i.e. there were no clusters with lower relative rCMR_{glc} in the A1+ group and higher relative rCMR_{glc} in the A1− group). Within group analyses were conducted to aid interpretation of the interactions.

** Statistically significant after applying a Bonferroni correction for 24 comparisons ($.05 / 24 = 0.002$).

Table 2

Ethanol effect on rCMRglc covaried with composite Anxiety/Fatigue score.

Cluster-level										Voxel-level		
		Volume Corrected	Cluster Size	ROI Size	%ROI	Volume Corrected	Coordinates					
		p Value	(voxels)		p<.005	p Value	T	x	y	z		
Main effects: Positive covariation												
ACC	Left	0.05	62	1067	6%	0.058	5.00	-10	44	-16		
	Right											
Thalamus	Left											
	Right	0.013	138	1073	13%	0.057	5.09	8	-28	8		
Main effects: Negative covariation												
Insula	Left	0.008	193	1519	15%	0.022	6.49	-34	14	2		
	Right											
Interaction: A1+ > A1-												
Insula	Left	0.038	93	1519	6%	0.034	5.98	-36	14	14		
	Right											
Ventral	Left	0.044	16	208	9%	0.018	4.75	-22	12	-12		
	Right											
Striatum												
A1+ Group: Positive covariation												
Amygdala	Left	0.021	31	144	22%	0.001**	8.20	-18	0	-14		
	Right	0.048	7	159	17%	0.006	5.62	26	-6	-14		
Dorsal	Left	0.003	254	993	31%	0.012	6.96	-12	-4	18		
	Right	0.020	130	1015	17%	0.040	5.67	12	4	18		
Ventral	Left	0.008	89	208	43%	0.003	6.55	-24	14	-10		
	Right											
Striatum	Left	0.004	221	1260	27%	0.032	5.90	-10	-4	14		
	Right	0.001**	309	1073	29%	0.010	7.20	12	-28	0		
A1+ Group: Negative covariation												
Insula	Left	0.005	230	1519	15%	0.003	9.49	-42	22	2		

Cluster-level				Voxel-level			
	Volume Corrected p Value	Cluster Size	ROI Size (voxels)	%ROI p<.005	Volume Corrected p Value	T	Coordinates x y z
Insula							
	Right						
	A1- Group: Negative covariation						
	Left	111	1519	7%	0.032	6.05	-36 14 14
	Right						

Clusters of voxels within the regions of interest (see table 1) where effects of ethanol on rCMRglc covaried with effects on a composite score of self-rated Anxiety and Fatigue (all voxel $p < 0.005$, volume-corrected spatial extent $p < 0.05$). All ROI interactions represent more positive covariation in the A1+ group, or more negative covariation in the A1- group. Below the interactions, planned comparisons assessed all ROIs for each allelic group.

** Statistically significant after applying a Bonferroni correction for 24 comparisons ($.05 / 24 = .002$).