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# Identifying Neurobiological Phenotypes Associated with Alcohol Use Disorder Severity

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Although numerous studies provide general support for the importance of genetic factors in the risk for alcohol use disorders (AUDs), candidate gene and genome-wide studies have yet to identify a set of genetic variations that explain a significant portion of the variance in AUDs. One reason is that alcohol-related phenotypes used in genetic studies are typically based on highly heterogeneous diagnostic categories. Therefore, identifying neurobiological phenotypes related to neuroadaptations that drive the development of AUDs is critical for the future success of genetic and epigenetic studies. One such neurobiological phenotype is the degree to which exposure to alcohol taste cues recruits the basal ganglia, prefrontal cortex, and motor areas, all of which have been shown to have a critical role in addictive behaviors in animal studies. To that end, this study was designed to examine whether cue-elicited responses of these structures are associated with AUD severity in a large sample ($n = 326$) using voxelwise and functional connectivity measures. Results suggested that alcohol cues significantly activated dorsal striatum, insula/orbitofrontal cortex, anterior cingulate cortex, and ventral tegmental area. AUD severity was moderately correlated with regions involved in incentive salience such as the nucleus accumbens and amygdala, and stronger relationships with precuneus, insula, and dorsal striatum. The findings indicate that AUDs are related to neuroadaptations in these regions and that these measures may represent important neurobiological phenotypes for subsequent genetic studies.
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## INTRODUCTION

Although behavioral genetic research clearly supports the importance of genetic factors in the risk for alcohol use disorders (AUDs; Agrawal and Lynskey, 2008; Heath *et al*, 1999; Knopik *et al*, 2004), identifying loci that contribute to the heritability of alcohol dependence has proven to be difficult (Dick and Foroud, 2003; Bierut *et al*, 2010; Treutlein *et al*, 2009). While difficulty in characterizing the genetic basis of AUDs can be attributed to multiple factors, one important consideration is that alcohol-related phenotypes used in genetic studies often reflect broadly defined diagnostic categories that yield highly heterogeneous subgroups (Hutchison, 2010). To address this issue, behavioral scientists have advocated an alternative approach that emphasizes the characterization of specific, narrowly defined intermediate phenotypes, or endophenotypes (Cannon and Keller, 2006; Gottesman and Gould, 2003). Large-scale studies suggest that use of intermediate phenotypes improve the ability to detect genetic factors that are associated with substance use disorders, including AUDs (Dick *et al*, 2006). Notably, recent reviews of this literature have called for efforts to develop intermediate neurobiological phenotypes, which could promote greater correspondence between human and animal models (Crabbe *et al*, 2010; Hutchison, 2010; Meyer-Lindenberg and Weinberger, 2006).

One potentially useful intermediate phenotype for AUDs is the degree to which exposure to alcohol cues recruits critical brain structures. This phenotype has been studied using animal models (eg, Rodd *et al*, 2004) as well as human neuroimaging studies (Filbey *et al*, 2008; George *et al* 2001; Hommer 1999; Wrase *et al*, 2007). One advantage of using cue-elicited changes in brain activation is the extensive animal literature, which provides a strong foundation for understanding the neurobiological mechanisms underlying the motivation to consume alcohol. The primary neural structures that have been identified as being critical for development of drug-seeking behavior in animal studies include the basolateral and central nucleus of the amygdala (Janak and Chaudhri, 2010; McBride, 2002), the medial prefrontal cortex (Carlson and Stevens, 2006), ventral tegmental area (VTA) (Stuber *et al*, 2008) and nucleus

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accumbens (NAc; Chaudhri *et al*, 2009; Knapp *et al*, 2009; Kelley, 2004), ventral pallidum (Harvey *et al*, 2002) and dorsal striatum (Wang *et al*, 2007), and the orbitofrontal cortex (OFC; Hansson *et al*, 2008). Alcohol and other drug-related cues acquire motivational significance over repeated learning trials through the strengthening of connections among these regions (for reviews see, Koob and Volkow, 2010; Koob, 2008; Schultz, 2007). As a result, approach behaviors become more likely after presentation of alcohol and other drug-related cues.

The search for similar neural mechanisms in human beings was difficult until the advent of functional neuroimaging. Neuroimaging studies typically use tasks in which neural response to a drug cue is compared with response during a neutral, non-drug-related cue. For example, visual alcohol-related cues (eg, pictures of beer or wine) elicit increased responses in NAc, anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), OFC, hippocampus, and insula compared with neutral non-alcohol-related cues (Lingford-Hughes *et al*, 2006; Myrick *et al*, 2004; Grusser *et al*, 2004; Ihssen *et al*, 2011). Presentation of the smell of one's favorite alcoholic beverage elicits enhanced response in NAc, dorsal striatum, occipital and parietal cortex, insula, and DLPFC (Kareken *et al*, 2004; Schneider *et al*, 2001). Finally, tastes of small amounts of alcoholic beverages have been found to elicit responses in ACC, DLPFC, OFC, insula, striatum, and thalamus (Filbey *et al*, 2008). Thus, in large part, human neuroimaging studies have replicated animal studies.

Although the above studies have provided information regarding the neural correlates of responses to alcohol cues, few of these studies (eg, Filbey *et al*, 2008) have reported associations of cue-elicited responses with severity of AUDs, and those that did often were limited to small sample sizes and a restricted range of alcohol use severity (eg, Filbey *et al*, 2008; George *et al*, 2001). This is an important question, as it is critical to identify neurobiological phenotypes (eg, BOLD response to alcohol taste cues) that are associated with clinical phenotypes (eg, loss of control over drinking) to better understand the development of AUDs. To address these issues, this study employs a large, diverse sample of heavy drinking individuals to identify the neurobiological phenotypes that are most strongly related to AUDs. In addition, this study examined demographic variables that are likely to influence these phenotypes, such as gender, treatment-seeking status, and smoking status. It was hypothesized that increased activation of ventral striatum, VTA, amygdala, OFC, DLPFC, and ACC would be associated with a greater history of alcohol exposure (ie, years of regular drinking) and greater AUD severity. We also hypothesized that BOLD response to alcohol cues would be greater in men, smokers, and treatment seekers.

## MATERIALS AND METHODS

### Participants

In all, 326 heavy drinking individuals (98 women; 30%) were included in this study. With institutional IRB approval, participants were recruited from the greater Albuquerque metropolitan region through advertisements placed in local print, online media, and radio advertisements to participate in two different translational studies evaluating alcohol use across heavy drinking treatment-seeking and non-treatment-seeking samples. This study focuses on the baseline relationship between alcohol use symptoms and brain response, and is therefore limited to the baseline behavioral and neuroimaging assessments from both studies. Treatment-seeking participants had not initiated psychosocial or pharmacological treatment at the time of the scanning session. During the baseline assessments, participants completed questionnaire data and a neuroimaging session that lasted approximately 2 h. Participants were compensated US$120 for their efforts. To participate in these studies, participants had to drink at least 5 or more drinks per drinking occasion for men (4 or more for women) at least five times in the past month. Participants were excluded if they reported a history of severe alcohol withdrawal, previous injury to the brain, or loss of consciousness for more than 5 min. In addition, all women were required to test negative on a pregnancy test administered before entering the MRI suite. All participants were required to have a breath alcohol concentration of 0.00 as measured by a breathalyzer before their participation in the scanning and assessment session and could not be in need of medical detoxification, as assessed by a score greater than eight on the Clinical Institute Withdrawal Assessment of Alcohol Scale, Revised (CIWA-Ar; Sullivan *et al*, 1989).

### Materials

To evaluate demographic variables, alcohol use and severity, and other substance use (including tobacco), the following questionnaires were administered: a demographics questionnaire, the Alcohol Use Disorder Identification Test (AUDIT) (Babor *et al*, 2001), the Alcohol Dependence Scale (ADS) (Skinner and Horn, 1984), the Time-Line Follow-Back (TLFB) for quantity and frequency of alcohol, cigarettes, and marijuana (Sobell and Sobell, 1992), the Failed Control subscale of Impaired Control Scale (ICS) (Heather *et al*, 1998; Heather *et al*, 1993), and a questionnaire to assess years of regular drinking.

### Alcohol Taste Cue Task

To measure cue-elicited responses to alcohol, we used a task described previously in our lab (Filbey *et al*, 2008; Hutchison *et al*, 2008). Participants received small amounts of two different beverages, pseudorandomly presented through Teflon tubing to the participant while they were in the scanner by a computer-controlled gustometer. The two beverages included small amounts (eg, 1 ml) of their preferred alcoholic beverage (eg, wine, alcohol, mixed drinks) alternated with a control beverage (litchi juice) that was selected for its appetitive and novel qualities. Each trial within the task began with a 'Ready' prompt for 2 s that designated the start of the trial, followed by taste cue presentation for 24 s, during which participants were instructed to Taste Alcohol (or Juice; seconds 1–10 and 12–22) and Swallow (seconds 10–12, 22–24). The taste cue presentation was followed by a washout period during which no taste stimuli were presented and participants viewed the word Rest for 16 s. Participants completed six trials of each tastant in each of two 9 min runs.

### Image Acquisition

All MRI data was collected on a 3T Siemens Trio (Erlangen, Germany) whole body scanner. Participants were placed in the scanner and a piece of tape was placed across the forehead to serve as feedback for movement reduction.

Before the acquisition of anatomical scans, localizer scans were acquired. An echo-planar gradient-echo pulse sequence (TR = 2000 ms, TE = 29, flip angle = 75°) was acquired with an 8-channel head coil, and images were acquired parallel to the ventral surface of a participant's OFC to reduce signal dropout and distortion in this region (Deichmann *et al*, 2003). Each volume acquired consisted of 33 axial slices ($64 \times 64$ matrix, $3.75 \times 3.75\,mm^2$, 3.5 mm thickness, 1 mm gap). In addition, a high resolution T1-weighted MP-RAGE anatomical image was acquired (TR = 2530 ms, TE = 1.64 ms, flip angle = 7°, 192 sagittal slices, $256 \times 256$ matrix, slice thickness = 1 mm, no gap) for each participant.

### Image Analysis

All analyses were completed using tools from the FMRIB Software Library (FSL). The first seven volumes of each functional run were discarded to allow the magnet to reach steady state. Motion Correction using FMRIB's Linear Image Registration Tool (Jenkinson *et al*, 2002) was used to realign images to the first volume within a run. Images were deskulled using BET, spatially smoothed with an 8 mm full-width half-max Gaussian kernel, temporally filtered using a high-pass filter of 100 s, and grand mean intensity normalized; all of these steps were performed using FMRIB Expert Analysis Tool (FEAT; Smith *et al*, 2004). Regressors of interest were created for the following conditions: alcohol cue, alcohol baseline, alcohol urge, juice cue, juice baseline, and juice urge, according to the timing scheme used in previous studies (Filbey *et al*, 2008; Hutchison *et al*, 2008). The primary contrast of interest reported in this study compared the alcohol cue *vs* juice cue. Statistical analyses were performed using the general linear model as implemented in FEAT. Customized square waveforms representing the condition of interest and the duration of stimulus presentation were convolved with a double gamma hemodynamic response function. Time-series analyses were conducted using FMRIB Improved Linear Model (Woolrich *et al*, 2004) with local autocorrelation estimation. This first-level analysis generated parameter estimates for each condition of interest, and contrast maps were computed for each participant. Contrast maps were registered to the Montreal Neurological Institute (MNI) 152-brain template using a two-step registration process with FMRIB Linear Image Registration Tool (FLIRT; Jenkinson *et al*, 2002). First, an average EPI image was registered to the participant's high-resolution anatomical image. Each participant's high-resolution T1-weighted image was then registered to the MNI 152-brain template. Finally, contrast maps were registered to the MNI 152-brain template using parameters from the previous registration steps.

Individual runs were combined within subjects using a fixed-effects model. The results from the second-level analyses were then used in a third-level group analysis using FMRIB Local Analysis of Mixed Effects (Woolrich *et al*, 2004) stage 1 only. Before computing group level statistics, all second-level contrast images were registered to the MNI template using parameters from the two-step registration process described above. For examinations of continuous variables (ie, AUDIT, ADS, ICSFC, years of regular alcohol use), we used linear regression to determine the correlation between the alcohol scale of interest and the contrast map of alcohol *vs* juice. To examine group differences as a function of gender, smoking status, and treatment-seeking status, we performed independent group *t*-tests on the contrast maps. Participants were considered smokers if they reported any cigarette smoking in the past 60 days. All group maps were masked to only examine gray matter. To protect against multiple comparison problems, group level statistical maps were thresholded using Gaussian random field theory (Worsley *et al*, 1992, 1996) as implemented in cluster-based thresholding in FSL. For each analysis, we used a voxelwise threshold of $z = 3.09$ and cluster threshold of $p < 0.05$ (In addition, all analyses were repeated using a voxelwise threshold of $z = 2.3$ and cluster level significance level of $p < 0.05$, to show the extent of the activation at standard statistical thresholds. Images for each of these analyses can be found in Supplementary Figures S1–S6.) In addition, the main effect analysis comparing alcohol to litchi was thresholded using a voxelwise threshold of $z = 8$, $p < 7 \times 10^{-16}$.

### Regions of Interest Analysis

In addition to our whole brain analysis, we also examined bilateral regions of interest (ROI) previously shown to be associated with enhanced response to alcohol cues to determine if these regions were associated with AUD severity including amygdala, NAc, VTA, DLPFC, OFC, and ACC. For the OFC, amygdala, and NAc ROIs, we used the Harvard Oxford probabilistic atlas in FSL to identify voxels within each ROI. The DLPFC ROI was a sphere with a radius of 5 mm around the DLPFC peak reported by Tapert *et al.* (2004). VTA was defined using a sphere with a radius of 2.5 mm around the coordinate $x = 0$, $y = -16$, and $z = -8$ (D'Ardenne *et al*, 2008). ACC was defined using a sphere with a 5 mm radius around the maximum response in ACC ($x = -6$, $y = 30$, $z = 14$) reported by Filbey *et al.* (2008). ROIs were then used as masks in the group level and covariate analyses; in each case, statistical maps were thresholded using Gaussian random field theory (Worsley *et al*, 1992, 1996) as implemented in cluster-based thresholding in FSL (voxel threshold $z = 2.3/p = 0.01$; cluster threshold $p < 0.05$).

## RESULTS

As shown by responses on the SCID and TLFB (see Table 1), this sample provided a wide range of AUD severity. As expected, treatment seekers showed higher scores on measures related to AUD severity such as the AUDIT ($t(322) = 12.22$, $p < 0.001$), the ADS ($t(323) = 9.74$, $p < 0.001$), the ICSFC scale ($t(324) = 13.78$, $p < 0.001$), and years of regular drinking ($t(324) = 14.44$, $p < 0.001$). A greater proportion of non-treatment seekers smoked marijuana ($\chi^2 = 12.96$, $p < 0.001$) than treatment seekers, but the two groups did not differ on cigarette smoking ($\chi^2 = 2.74$, $p = 0.09$). As

**Table 1** Sample Characteristics

| Gender | Treatment seeking | | | |
|---|---|---|---|---|
| | Yes | | No | |
| | Male | Female | Male | Female |
| *n* | 96 | 51 | 130 | 49 |
| Age | 39.0 (9.1) | 40.6 (8.8) | 26.1 (4.6) | 24.3 (2.5) |
| ADS total | 17.1 (8.4) | 19.3 (9.0) | 10.2 (5.8) | 9.4 (5.7) |
| AUDIT | 23.3 (6.4) | 24.6 (7.7) | 15.6 (6.0) | 13.6 (5.4) |
| ICS-FC | 15.1 (6.7) | 13.9 (7.6) | 26.1 (8.1) | 26.6 (7.8) |
| Yrs drinking | 20.2 (9.8) | 19.4 (9.6) | 8.4 (5.5) | 6.6 (3.5) |
| Avg. drinks | 9.6 (5.5) | 8.0 (4.3) | 6.3 (2.4) | 4.9 (2.1) |
| Drinking days (30) | 20.0 (8.3) | 19.9 (7.8) | 16.0 (6.5) | 14.1 (6.1) |
| % Cig smokers | 39.6 | 51.0 | 35.4 | 34.7 |
| Avg. cigs | 11.1 (7.3) | 12.1 (8.2) | 11.7 (7.5) | 7.6 (5.9) |
| % MJ smokers | 29.5 | 20.0 | 46.5 | 45.8 |
| % Days smoked MJ | 24.1 (25.7) | 26.5 (38.7) | 30.0 (35.6) | 38.0 (38.8) |
| CIWA range (mean) | 0–7 (1.56) | 0–5 (1.68) | — | — |

ADS—Alcohol Dependence Scale; AUDIT—Alcohol Use Disorder Identification Test; ICS-FC—Impaired Control Scale, Failed Control subscale; Yrs drinking—number of years of regular drinking; Avg. drinks—average number of standard drinks consumed per drinking day; drinking days (30)—number of drinking days in the past 30 days; % Cig smokers—percentage of participants who reported smoking in the past 60 days; Avg. cigs—number of cigarettes smoked per smoking day; % MJ smokers—percentage of participants who reported smoking marijuana at least one time per month; % days smoked MJ—percentage of days that marijuana was smoked.
Means (SD) for measures collected during the baseline questionnaire session.

**Table 2** Correlations between Alcohol Severity and Exposure Variables

| | AUDIT | ADS | ICS-FC | Yrs drinking |
|---|---|---|---|---|
| AUDIT | — | 0.82 | 0.78 | 0.44 |
| ADS | — | — | 0.70 | 0.34 |
| ICS-FC | — | — | — | 0.39 |

ADS—Alcohol Dependence Scale; AUDIT—Alcohol Use Disorder Identification Test; ICS-FC—Impaired Control Scale, Failed Control subscale; Yrs drinking—number of years of regular drinking.
All $p$'s $<0.00001$.

expected, our covariates of interest were highly intercorrelated (see Table 2). Across all participants, 51% received beer, 40% received spirits, and 9% received wine as the alcohol-containing beverage during the taste task.

## Main Effects of Alcohol Cues *vs* Control Cues

As seen in Figure 1, across all participants, the contrast of alcohol *vs* juice shows a difference that encompasses one large cluster that includes the entire striatum, thalamus, medial frontal cortex (ACC, dorsomedial PFC, supplementary motor area), brainstem, bilateral OFC, bilateral insula, amygdala, and cerebellum. Peak detection algorithms showed that peaks of activation differences appear in ACC (BA 24), right lateral OFC, bilateral anterior insula, bilateral amygdala, bilateral caudate head, thalamus, putamen, VTA, posterior cingulate cortex, and cerebellum (see Figure 1 and Table 3). In addition, in the ROI analysis, we found significant effects in all ROIs, except right DLPFC (see Table 4).

## Covariate Analyses

*Audit.* In the whole brain analysis, positive correlations between the AUDIT and the contrast of alcohol *vs* juice emerged in precuneus, posterior insula, posterior cingulate cortex, globus pallidus, and putamen (see Figure 2a and Table 3). ROI analyses showed positive significant relationships with AUDIT scores in left NAc, left OFC, left DLPFC, and right amygdala (see Table 4).

*Alcohol dependence scale.* In the whole brain analysis, positive correlations between the ADS and the contrast of alcohol *vs* juice emerged in cuneus, precentral gyrus, and fusiform gyrus ($z>3.09$, $p<0.05$) (see Figure 2b and Table 3). ROI analyses showed significant positive correlations with ADS in ACC, left DLPFC, left OFC, bilateral NAc, and right amygdala (see Table 4).

*ICS—failed control.* Our whole brain analysis showed that failures in control over drinking were positively associated with the alcohol–juice contrast in the caudal portion of the ACC extending into the supplementary motor area, bilateral pre/postcentralgyrus, bilateral insula, bilateral putamen, thalamus, and globus pallidus, parahippocampal gyrus, precuneus, and brainstem (see Figure 2c and Table 3; $z>3.48$, $p<0.05$). ICSFC showed significant positive relationships with several ROIs including bilateral amygdala, left DLPFC, and left NAc (see Table 4).

*Years of regular drinking.* Whole brain analyses of the correlation between self-reported years of regular drinking and BOLD responses in the alcohol–juice contrast showed significant relationships in precuneus/lateral occipital cortex, and cuneus (see Figure 2d and Table 3). ROI analyses with years of regular drinking showed significant effects bilateral DLPFC and left NAc (see Table 4).

*Multiple regression analyses.* Because all our independent variables measuring severity of AUDs were highly correlated, we examined the independent influence of each variable to the overall alcohol *vs* juice contrast by entering each variable in a multiple regression analysis in which each independent variable was orthogonalized with respect to each other intravenously. In the multiple regression analysis, AUDIT showed no significant effects that met cluster level thresholding. In contrast, ICSFC showed a positive relationship with the alcohol–litchi contrast in a cluster that included right lateralized central operculum/insula, precentral gyrus, and putamen. ADS showed a positive relationship in inferior precuneus/cuneus. Finally, years of regular drinking showed a positive relationship with inferior and superior precuneus and lateral occipital cortex.

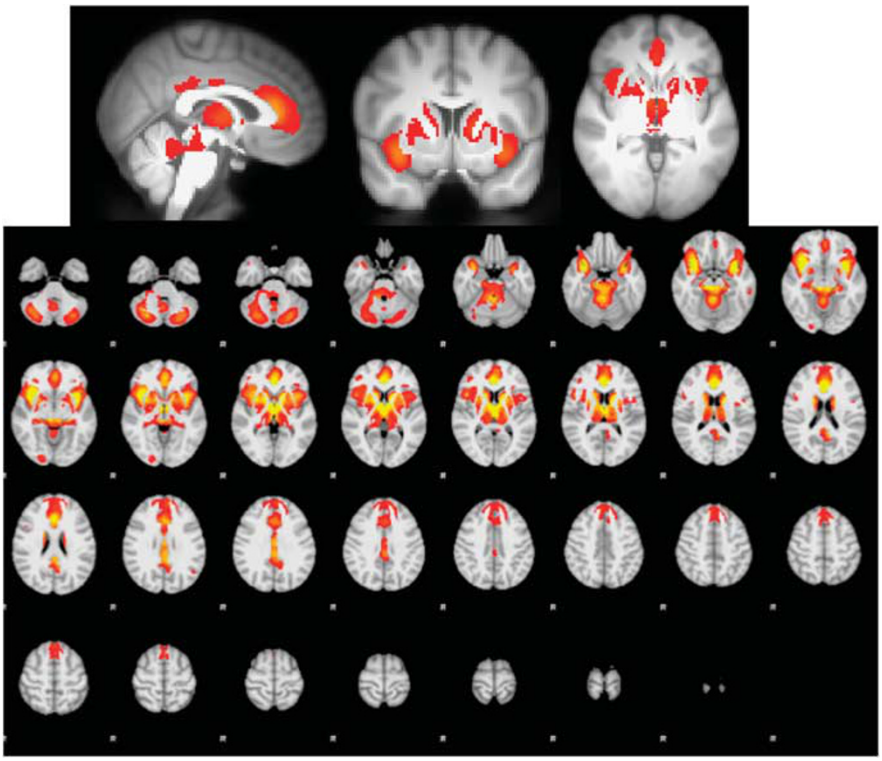

**Figure 1** Main effect of blood oxygen level-dependent (BOLD) response to alcohol cues−litchi cues, cluster corrected at $z > 8$, $p < 0.05$, for visualization purposes. The comparison of alcohol cues to litchi cues showed significant differences in anterior and posterior cingulate cortex, dorsal striatum, insula, thalamus, and brainstem.

### Group Level Comparisons

*Gender.* No significant differences were found when comparing responses to alcohol *vs* litchi across men and women in either whole brain analysis. However, in the ROI analysis, men showed enhanced response in the left amygdala compared with women in the alcohol−litchi contrast. This relationship remained even after controlling for ADS scores.

*Smoking.* No significant differences emerged between cigarette smokers and non-smokers in the alcohol−juice contrast in the whole brain analysis. However, the ROI analysis showed differences in bilateral NAc, such that non-smokers had a greater difference between alcohol and litchi. However, given that non-smokers scored higher on AUD severity than smokers, we covaried ADS scores and found that the NAc findings were no longer significant, suggesting that AUD severity was driving the effect.

*Treatment-seeking status.* Compared with non-treatment seekers, treatment-seeking subjects showed significantly greater response in precuneus, middle temporal gyrus, inferior temporal gyrus, and SMA. In contrast, non-treatment seekers did not show any significantly greater responses in the contrast of alcohol−litchi compared with treatment seekers (see Figure 2e and Table 3). In the ROI analysis, treatment seekers showed greater response than non-treatment seekers in left DLPFC, left NAc, and left amygdala (see Table 4).

## DISCUSSION

Exposure to the taste of alcohol as compared with an appetitive control taste cue resulted in widespread response throughout regions important for incentive motivation (Arana *et al*, 2003; McClure *et al*, 2003; Robinson and Berridge, 2003) and planned motor behavior (Rushworth *et al*, 2004). Specifically, we found maximal differences in ACC, bilateral OFC/insula, dorsal striatum, amygdala, thalamus, VTA, and cerebellum when comparing neural responses to the delivery of alcohol and litchi juice. Additional differences appeared in bilateral ventral striatum and left DLPFC in the ROI analyses. These findings were not surprising, given the myriad studies investigating cue-elicited responses to alcohol that have shown similar effects (Filbey *et al*, 2008; George *et al* 2001; Kareken *et al* 2004; Wrase *et al*, 2007). Although previous studies have primarily focused on ventral striatal responses to alcohol cues, this study suggests that dorsal striatum has an integral role in addictive behavior Vollstadt-Klein *et al*, 2010). The dorsal striatum contributes significantly to habit learning (Belin *et al*, 2009; Costa, 2007), an expected consequence of repeated ingestion of rewarding substances such as alcohol (Wise, 2009; Koob and Volkow, 2010). Dorsal striatal circuits project and interact with supplementary motor areas involved in motor responses and planning. Specifically, it is believed that GABAergic cell bodies in the dorsal striatum project to the globus pallidus, and activation of these cells results in a disinhibition of the thalamus, allowing sensory input to flow to motor control regions of

**Table 3** Cluster Sizes and Locations of Significant Clusters in all Neuroimaging Analyses

| Contrast | Brain region | BA | Max z | Mean r (max r) | Voxels | x | y | z |
|---|---|---|---|---|---|---|---|---|
| Main effect | Inferior frontal gyrus (L) | 13 | 11.7 | — | 3160 | −34 | 10 | −14 |
| | Extra-nuclear (R) | 13 | 12 | — | 1224 | 36 | 12 | −12 |
| | Anterior cingulate (L) | 24 | 12.6 | — | 861 | 0 | 30 | 10 |
| | Cingulate gyrus (L) | 23 | 9.27 | — | 273 | 0 | −16 | 28 |
| | Thalamus (L) | | 8.62 | — | 36 | −20 | −20 | 10 |
| | Cerebellum (R) | | 8.02 | — | 4 | 32 | −70 | −36 |
| | Cerebellum (L) | | 8.08 | — | 2 | −26 | −70 | −34 |
| | Caudate (R) | | 8.03 | — | 1 | 18 | −8 | 22 |
| AUDIT | Precuneus (R) | 31 | 4.46 | 0.20 (0.24) | 1151 | 16 | −58 | 22 |
| | Insula (R) | 13 | 4.52 | 0.20 (0.24) | 878 | 48 | −34 | 24 |
| | Paracentral lobule (R) | 5 | 4.34 | 0.19 (0.23) | 781 | 8 | −36 | 46 |
| | Inferior parietal lobule (L) | 40 | 4.47 | 0.19 (0.23) | 748 | −50 | −36 | 30 |
| | Parahippocampal gyrus (R) | 35 | 4.23 | 0.19 (0.23) | 376 | 20 | −20 | −14 |
| | Putamen/pallidum (L) | | 4.09 | 0.19 (0.23) | 320 | −18 | −12 | −4 |
| ADS | Cuneus (L) | 7 | 4.73 | 0.19 (0.24) | 2104 | −6 | −72 | 32 |
| | Precentral gyrus (L) | 6 | 4.8 | 0.19 (0.24) | 1679 | −62 | 4 | 6 |
| | Fusiform gyrus (L) | 19 | 4.78 | 0.19 (0.25) | 948 | −22 | −66 | −10 |
| | Cerebellum (L) | | 3.89 | 0.18 (0.21) | 397 | −22 | −56 | −38 |
| ICSFC[a] | Insula (R) | 13 | 4.91 | 0.21 (0.25) | 2362 | 48 | −36 | 22 |
| | Inferior parietal lobule (L) | 40 | 5.17 | 0.21 (0.25) | 2255 | −48 | −38 | 28 |
| | Precuneus (L) | 7 | 5.14 | 0.21 (0.26) | 1494 | −12 | −52 | 48 |
| | Brainstem (L) | | 4.77 | 0.21 (0.24) | 1341 | −10 | −24 | −30 |
| | Parahippocampal gyrus (R) | 35 | 5.23 | 0.21 (0.25) | 987 | 20 | −20 | −12 |
| | Medial frontal gyrus (L) | 6 | 4.76 | 0.21 (0.25) | 736 | −8 | −2 | 54 |
| | Precuneus (R) | 31 | 5.24 | 0.22 (0.26) | 482 | 22 | −68 | 22 |
| | Uncus (L) | 36 | 4.29 | 0.21 (0.23) | 179 | −18 | −6 | −38 |
| Yrs drinking[a] | Precuneus (R) | 19 | 4.68 | 0.21 (0.24) | 1620 | 30 | −76 | 32 |
| | Cuneus (L) | 19 | 4.46 | 0.21 (0.24) | 570 | −10 | −78 | 32 |
| Tx > non-Tx | Precuneus (L) | 7 | 5.02 | — | 3572 | −10 | −54 | 48 |
| | Middle temporal gyrus (L) | 22 | 4.38 | — | 1432 | −48 | −46 | 2 |
| | Inferior temporal gyrus (L) | 20 | 4.26 | — | 854 | −44 | −14 | −28 |
| | Medial frontal gyrus (L) | 6 | 4.05 | — | 343 | −4 | −8 | 54 |

AUDIT—Alcohol Use Disorder Identification Test; ADS—Alcohol Dependence Scale; ICS-FC—Impaired Control Scale, Failed Control subscale; Yrs drinking—number of years of regular drinking; Tx—treatment seeking; non-Tx—on-treatment seeking.
[a]Images thresholded at $z > 3.48$, $p < 0.05$ cluster corrected. Mean (max) *r*—mean (max) correlation coefficient within the significant cluster.

frontal cortex (Alexander and Crutcher, 1990; Alexander *et al*, 1990; Wickens, 1997). Given that we found both dorsal striatum and presupplementary motor area in our main contrast, this may suggest that functional connectivity between these regions is enhanced as a result of habit learning. Future studies are needed to investigate the functionally connected networks that contribute to craving responses.

In addition to the main contrast of alcohol and litchi, we were also interested in how these differences correlated with measures of AUD severity. Commonalities among the correlations between clinical measures and brain responses included significant relationships with signal change in left NAc, left DLPFC, and amygdala in the ROI analyses. Similar correlation patterns with severity measures also occurred in the precuneus and globus pallidus, insula, parahippocampal gyrus, and ACC/SMA in the whole brain analyses. The amygdala and NAc are two important structures for assigning value to emotional stimuli, and left DLPFC may be important for directing attention towards rewarding stimuli (Savine and Braver, 2010). The basolateral amygdala and NAc, along with frontal cortex, are part of a final common pathway for cue-elicited craving and relapse (Kalivas and Volkow, 2005; Koob and Volkow, 2010).

Although the amygdala/NAc results confirm previous models and findings, this study adds to the previous literature by suggesting a prominent role of precuneus and insula in cue reactivity in more severe AUDs. While previous studies have shown evidence of the role of precuneus in cue reactivity (Tapert *et al*, 2004; Myrick *et al*, 2008), structures within the mesolimbic dopamine system have received the most focus. However, the insula has received increasing

**Table 4** Relationships between ROIs and Clinical Assessment Variables

| Measure | Brain region | Max *z* | Mean *r* (max *r*) | Voxels | *x* | *y* | *z* |
|---|---|---|---|---|---|---|---|
| Main effect | ACC | 12.3 | — | 81 | −2 | 28 | 12 |
| | Left amygdala | 9.68 | — | 299 | −32 | 0 | −16 |
| | Left DLPFC | 3.98 | — | 14 | −18 | 22 | 50 |
| | Left OFC | 9.99 | — | 997 | −30 | 10 | −18 |
| | Left NAc | 7.61 | — | 48 | −8 | 6 | −4 |
| | Right amygdala | 10.1 | — | 299 | 32 | 2 | −16 |
| | Right OFC | 11.2 | — | 915 | 38 | 18 | −12 |
| | Right NAc | 7.31 | — | 57 | 10 | 10 | −4 |
| | VTA | 7.89 | — | 7 | 0 | −16 | −6 |
| AUDIT | Left DLPFC | 3.22 | 0.15 (0.178) | 11 | −18 | 18 | 50 |
| | Left OFC | 3.55 | 0.15 (0.189) | 122 | −36 | 32 | 0 |
| | Left NAc | 2.69 | 0.14 (0.149) | 20 | −10 | 16 | −8 |
| | | 2.42 | 0.13 (0.134) | 4 | −6 | 6 | −6 |
| | | 2.31 | 0.13 (0.128) | 1 | −6 | 16 | −4 |
| | Right amygdala | 3.61 | 0.15 (0.199) | 41 | 24 | −8 | −10 |
| ADS | ACC | 2.43 | 0.13 (0.135) | 2 | −8 | 26 | 12 |
| | Left DLPFC | 3.11 | 0.15 (0.172) | 19 | −18 | 18 | 52 |
| | | 2.37 | 0.13 (0.131) | 2 | −26 | 18 | 50 |
| | Left OFC | 3.59 | 0.15 (0.185) | 172 | −40 | 30 | −2 |
| | Left NAc | 3.15 | 0.15 (0.174) | 46 | −14 | 16 | −6 |
| | Right amygdala | 3.15 | 0.14 (0.174) | 32 | 24 | −10 | −10 |
| | Right NAc | 2.34 | 0.13 (0.13) | 1 | 8 | 18 | −2 |
| | | 2.32 | 0.13 (0.128) | 1 | 6 | 14 | −4 |
| ICSFC | Left amygdala | 3.89 | 0.16 (0.207) | 223 | −22 | −10 | −12 |
| | Left DLPFC | 3.25 | 0.15 (0.179) | 11 | -18 | 18 | 50 |
| | Left NAc | 2.84 | 0.14 (0.157) | 50 | −12 | 8 | −8 |
| | Right amygdala | 3.92 | 0.16 (0.21) | 174 | 28 | 2 | −26 |
| Yrs drinking | Left DLPFC | 2.63 | 0.14 (0.145) | 9 | −20 | 18 | 52 |
| | Left NAc | 2.83 | 0.14 (0.157) | 11 | −12 | 6 | −10 |
| | Right DLPFC | 2.6 | 0.13 (0.144) | 4 | 24 | 18 | 48 |
| Male > female | Left Amygdala | 3.0 | — | 36 | −16 | −2 | −18 |
| Non-smoker > smoker | Left NAc | 3.74 | — | 39 | −12 | 10 | −6 |
| | Right NAc | 3.29 | — | 45 | 12 | 12 | −6 |
| Tx > non-Tx | Left amygdala | 3.54 | — | 47 | −30 | −8 | −20 |
| | Left DLPFC | 3.65 | — | 31 | −18 | 18 | 50 |
| | Left NAc | 3.79 | — | 56 | −12 | 6 | −10 |

AUDIT—Alcohol Use Disorder Identification Test; ADS—Alcohol Dependence Scale; ICS-FC—Impaired Control Scale, Failed Control subscale; Yrs drinking—number of years of regular drinking; Tx—treatment seeking; non-Tx—non-treatment seeking.
Images were thresholded at $z > 2.3$, $p < 0.05$ cluster corrected. Mean (max) *r*—mean (max) correlation coefficient within the significant cluster.

amounts of attention in the addiction literature since reports that damage to this region resulted in reduced levels of craving in smokers (Naqvi *et al*, 2007). The correlations of the insula with AUD severity measures suggests a potential increase in processing of interoceptive cues triggered by cue presentation (Bechara 2005; Critchley, 2005; Goldstein *et al*, 2009; Gray and Critchley, 2007; Lovero *et al*, 2009; Naqvi and Bechara, 2009; Paulus, 2007). The precuneus is functionally connected to the insula and also portions of the dorsal striatum (Marguiles *et al*, 2009), and these findings suggest that this functional circuit may be enhanced in individuals with more severe AUDs. Again, because we did not specifically investigate functional connectivity, this speculation must be tested in future studies. Regardless, given the role of the precuneus in maintaining attention and mental imagery (Cavanna and Trimble, 2006), the findings in this study suggest that this region may be important in automatic attentional biases towards alcohol-related cues (Sharma *et al*, 2001; Stetter *et al*, 1995; Stormark *et al*, 1997).

In addition to severity of use, we examined factors that have been previously shown to influence subjective craving

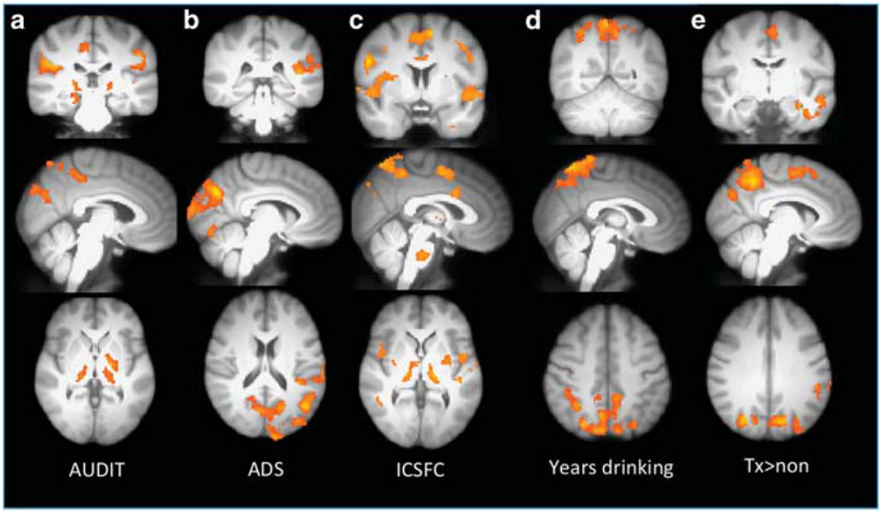


**Figure 2** Correlation of blood oxygen level-dependent (BOLD) response with measures of alcohol abuse severity. Examination of the correlation between the alcohol–litchi contrast image and four measures of alcohol use severity/alcohol exposure suggests that craving responses for alcohol cues engage regions associated with habit learning and motor control in more experienced drinkers and those who experience more problems as a result of alcohol use (a–d). (e) Differences in the alcohol *vs* litchi contrast between treatment seekers and non-treatment seekers. Treatment seekers showed greater differences between the two conditions in the precuneus, SMA, compared with non-treatment seekers. All images are threshold at $z = 3.09$, cluster corrected $p < 0.05$, except where noted. (a) AUDIT—Alcohol Use Disorder Identification Test; (b) ADS—Alcohol Dependence Scale; (c) ICS-FC—Impaired Control Scale, Failed Control subscale; (d) years of regular drinking; and (e) treatment seeking *vs* non-treatment seeking.

or neural responses to cues. These included factors such as gender, smoking status, and treatment-seeking status. The comparison of men and women revealed significant differences in the left amygdala, such that men had greater responses than women. These findings are consistent with previous studies that suggest men have a stronger affective response to rewarding stimuli (Hamann, 2005), and suggest that men found the alcohol more rewarding than the women. Smoking status influenced responses in bilateral NAc, but this effect was likely due to the fact that non-smokers had more severe AUDs, as measured by the AUDIT and ADS. When examining treatment seekers compared with non-treatment seekers, we found activation differences in regions that were largely consistent with those observed in the AUDIT and ICSFC analyses. In addition, treatment-seeking subjects showed greater response in ventral striatum in the ROI analysis. These findings are not surprising given that treatment seekers showed more severe dependence and more years of drinking compared with non-treatment seekers. Unexpectedly, treatment seekers actually showed greater activation of DLPFC than non-treatment seekers, a finding that is inconsistent with a previous review of cue-elicited craving that report DLPFC and OFC only in non-treatment seekers (Wilson *et al*, 2004). One potential difference between this study and those cited by Wilson *et al* (2004) is the number of subjects included in each analysis. Many previous studies of cue-elicited craving have used very small sample sizes, which may have limited the ability to detect smaller effects or resulted in spurious findings. However, this study includes a very large sample of treatment- and non-treatment-seeking subjects, and thus power and sample representativeness are not likely issues. These results suggest that frontally mediated craving responses are enhanced in individuals seeking treatment for alcohol dependence, and it may be possible that alcohol and associated cues usurp frontal control systems in the later stages of alcohol dependence that are used to obtain or plan out future drug seeking behavior. Alternatively, these frontal regions may be preferentially recruited in treatment seekers because they are actively trying to control urges to drink, given that these individuals participated in a study advertising treatment for alcohol dependence. Future studies are needed to adjudicate between these alternative hypotheses.

## Limitations and Conclusions

Although this study provides a sufficiently powered investigation into the neural correlates of cue-induced craving and the relationship with severity of alcohol problems, it is important to consider the findings in light of the following limitations. First, to participate, individuals were required to abstain from drinking. While none of our participants were in need of medical detoxification (as determined by the CIWA), it would be logical to assume that during the scan session, many may have experienced some level of withdrawal that may have contributed to the observed patterns of activation. As many studies with nicotine have shown, withdrawal can influence BOLD responses in ACC, striatum, posterior cingulate, and DLPFC (McBride *et al*, 2006; McClernon *et al*, 2008), suggesting that many of our correlations with severity may also be modulated by withdrawal state. Future studies will be needed that explicitly tease apart the effects of acute withdrawal and AUD severity on craving responses. Second, our treatment-seeking participants used less marijuana than our non-treatment participants, a potential confound when interpreting the results of this study. For example, recent cannabis use has been shown to decrease frontal responses under conditions of stress (Li *et al*, 2005), and the differences in marijuana

use between our treatment and non-treatment samples may have influenced frontal responses during the taste task.

In conclusion, this study has identified the core neural substrates that underlie responses to alcohol cues and identified specific regions that are related to the progression of alcohol abuse severity. Across all participants, alcohol cues, compared with a juice cue, significantly activated regions important for motivated behavior such as lateral OFC, anterior cingulate, ventral striatum, caudate, and putamen. In addition, AUD severity and years of regular drinking were correlated with regions involved in sensorimotor processing such as the precuneus and supplementary motor area. Overall, these results suggest that as individuals progress along a continuum of AUD severity, regions involved in motor behavior in addition to affective processing, become more potently engaged in response to alcohol-related cues. Greater recruitment of motor circuits may correspond with an increasing lack of control over alcohol use. The findings suggest that measures of functional changes in these regions may represent an important phenotype for genome-wide studies that are designed to identify genetic variation that underlies the progression of neuroadaptations in these regions. Finally, it is important to note that these phenotypes may also represent important targets for the development of new treatment approaches. For example, medications that disrupt the connection between the striatum and motor areas may be particularly useful for relapse prevention. Ultimately, these neurobiological phenotypes may eventually be useful for identifying genetic variations or brain measures that predict response to treatment (see Hutchison, 2010).

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