

Exposure to the Taste of Alcohol Elicits Activation of the Mesocorticolimbic Neurocircuitry

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A growing number of imaging studies suggest that alcohol cues, mainly visual, elicit activation in mesocorticolimbic structures. Such findings are consistent with the growing recognition that these structures play an important role in the attribution of incentive salience and the pathophysiology of addiction. The present study investigated whether the presentation of alcohol taste cues can activate brain regions putatively involved in the acquisition and expression of incentive salience. Using functional magnetic resonance imaging, we recorded BOLD activity while delivering alcoholic tastes to 37 heavy drinking but otherwise healthy volunteers. The results yielded a pattern of BOLD activity in mesocorticolimbic structures (ie prefrontal cortex, striatum, ventral tegmental area/substantia nigra) relative to an appetitive control. Further analyses suggested strong connectivity between these structures during cue-elicited urge and demonstrated significant positive correlations with a measure of alcohol use problems (ie the Alcohol Use Disorders Identification Test). Thus, repeated exposure to the taste alcohol in the scanner elicits activation in mesocorticolimbic structures, and this activation is related to measures of urge and severity of alcohol problems.

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INTRODUCTION

The construct of craving for substances of abuse by humans has often been defined as the strong desire or urge to consume substances, such as alcohol. Understanding the factors that lead to craving is important as a reduction in craving is often the target of behavioral and pharmacological interventions (Tiffany and Conklin, 2000; Anton, 1999; Singleton and Gorelick, 1998). However, there has been some controversy about the role and definition of craving and a rift exists between the way it is conceptualized in animal and human models (See, 2002). One area of agreement, however, is that theories of craving derived from the animal literature have emphasized the role of the mesocorticolimbic connections as the substrate underlying the attribution of incentive salience to cues associated with drug use (Robinson and Berridge, 2001).

Consistent with these findings, are recent studies with humans that employ neuroimaging approaches, as they also implicate mesocorticolimbic structures in the pathophysiol-

ogy of addiction (Kalivas and Volkow, 2005; Volkow *et al*, 2005). More specifically, this mesocorticolimbic circuitry subserves the attribution of incentive salience or motivation to stimuli that signal reward. For example, recent neuroimaging studies have demonstrated that food cues (eg taste) activate this mesocorticolimbic circuitry (Pelchat *et al*, 2004; O'Doherty *et al*, 2001). However, alcohol and drugs produce an even more powerful activation of this circuitry. As a result, excessive incentive salience becomes linked to cues associated with drug use and produces strong motivation to use drugs in the context of these cues (Kalivas and Volkow, 2005).

Consistent with the broader neuroimaging literature on addiction, mesocorticolimbic activation has been found with visual alcohol cues (Braus *et al*, 2001), olfactory cues (Kareken *et al*, 2004; Schneider *et al*, 2001), and an interesting combination of gustatory and visual cues (George *et al*, 2001; Myrick *et al*, 2004). In two related studies, a single taste 'cue' was presented before the actual scanning procedure in the form of a priming dose of an alcoholic taste (ie 1 Oz of beer) (Myrick *et al*, 2004; George *et al*, 2001). During scanning, pictures of alcoholic and non-alcoholic beverages were presented. These studies reported that after a priming dose of alcohol, visual cues elicit activation of primary reward areas, such as the striatum, ventral tegmental area (VTA), and anterior cingulate gyrus. As the alcohol taste cue was presented before any scanning

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data were collected and data immediately following the alcohol taste cue (ie first few volumes) were not analyzed in isolation, interpretation of BOLD activation patterns and subjective ratings are likely to reflect the response to the visual cues rather than the priming dose. Furthermore, as a baseline condition was not acquired before the priming dose, the specific effects of the taste cue on self-ratings or brain responses cannot be determined.

Hence, at the present time, the extent to which the gustatory effects of alcohol may elicit activation of the mesocorticolimbic circuitry is unclear. Given that gustatory cues associated with food and beverages elicit activation of this same circuitry (O'Doherty *et al*, 2001, 2002; Small *et al*, 2001; Frank *et al*, 2003;) and given the studies noted above, gustatory cues of alcoholic beverages may represent a means of activating these mesolimbic structures. Thus, the overarching objective of the present study was to examine the effect of gustatory alcohol cues presented, whereas participants were scanned on the activation of the mesocorticolimbic circuitry. More specifically, it was hypothesized that exposure to an alcoholic taste would activate structures involved in this circuitry (eg VTA, striatum, orbitofrontal cortex (OFC), prefrontal cortex) as compared with both a resting baseline and an appetitive control cue (juice taste).

A second objective of the study was to link the neuroimaging data to behavioral measures both at the time of testing and outside of the experimental context. To do so, we examined the correlations between the brain indices of mesocorticolimbic activation and urge during the task. In addition, we examined the correlation with a standardized index of alcohol-related consumption and problems (ie the Alcohol Use Disorders Identification Test (AUDIT)). We predicted that activation of mesocorticolimbic regions would predict these behavioral measures linked to substance use and abuse.

MATERIALS AND METHODS

Participants

Thirty-eight healthy volunteers who reported drinking alcohol frequently were recruited through advertisements (ie flyers and e-mail listings) and agreed to take part in the study, but only 37 were included in the analyses due to technical problems with one volunteer's data ($N=37$, 25 males, 12 females, mean age: 22.65) (Table 1). All participants were right-handed. Participants signed written informed consents approved by the University of Colorado Human Research Committee.

Measures

Quantity and frequency of alcohol use and problems related to alcohol use were measured by the AUDIT (Saunders *et al*, 1993). The Alcohol Urge Questionnaire (AUQ; Bohn *et al*, 1995) was used to measure current urge for alcohol (Table 1). A Demographics Questionnaire was used to collect general information about participants, such as years of education and gender. The Edinburgh Handedness Inventory (Oldfield, 1971) was used to assure that all participants were right-handed.

Table 1 Characterization of Participants

	Mean	SD
N	37	—
Age	22.60	2.12
Education	15.72	1.04
Average number of drinks per occasion	5.53	2.37
Largest number of drinks on one occasion	14.56	6.67
Average times per month drank alcohol	11.80	4.00
Number of times drank ≥ 5 drinks per occasion	7.61	4.59
Total AUDIT	12.80	5.34
Baseline AUQ total ^a	9.73	4.77
Post-scan AUQ total ^a	14.39	6.9

The study sample's demographic characteristics, alcohol use, AUDIT, and AUQ ratings are described in this table.

^aTotal AUQ score from 8–56, greater number indicating greater urge to drink alcohol.

Procedures

All scanning sessions took place at the University of Colorado Health Sciences Center's Brain Imaging Center between the hours of 0800 and 1600. Before the scanning session, participants were asked to abstain from alcohol for 24 h and to abstain from caffeine and cigarettes for the preceding 2 h. Participants were breathalyzed at the beginning of their session to ensure abstinence from alcohol. All participants began their experimental session by completing a battery of questionnaires that assessed urge and mood. Participants were fitted with vision correction lenses if needed and were oriented to the taste-cue paradigm and scanning procedures (ie liquid was delivered into their mouth via plastic tubing). After orientation with the taste procedure, participants completed two echo-planar imaging (EPI) runs lasting approximately 9 min each and a series of anatomical images described below. The participants were in the scanner for approximately 60 min (part of this time was spent on an unrelated paradigm described elsewhere). Upon completion of the scanning session, participants were asked to complete current mood, alcohol urge, and craving assessments.

Taste-Cue Paradigm

All taste stimuli were delivered to the participants via Teflon tubing using a computer-controlled delivery system as described by Frank *et al* (2003). The alcohol stimuli used were each subject's preferred alcoholic beverage, whereas the control stimulus was kept constant across subjects. We selected a control stimulus (ie litchi juice) in an attempt to provide an appetitive control for the activation of the mesocorticolimbic circuitry, given that previous studies suggest activation of this circuitry after juice cues (eg Berns *et al*, 2001). During the EPI run, there were 12 pseudo-randomized alcohol and control trials (six of each). Each trial consisted of a 24-s taste delivery period, followed by a washout period to allow the liquid taste to dissipate before the next trial. During a pilot study, we determined that 1 ml of liquid over 24 s provided the greatest taste detection, while still maintaining minimal head movement. The word

'TASTE' was visually presented throughout the taste period. In 13 of the 37 subjects, the words 'TASTE ALCOHOL' during the alcohol taste period and 'TASTE CONTROL' during the control taste period were visually presented. No difference in response to alcohol *vs* rest, litchi *vs* rest, and alcohol *vs* control was found between the groups with the explicit taste instructions ($N=13$) compared with the group with the non-explicit taste instructions ($N=24$). The washout period consisted of a 16-s rest period during which the word 'REST' appeared on the screen; nothing was delivered during the rest period. The washout was followed by a 2-s urge question and a 2-s prompt screen (Figure 1). During the urge question, the subjects were asked to rate their current subjective urge to drink alcohol using a scale of 1 (no urge at all) to 4 (very high urge).

fMRI Data Acquisition

The functional EPI images were acquired on a GE 3T scanner (Milwaukee, WI). As the OFC is involved in the craving/reward system and can suffer from severe signal dropout caused by susceptibility effects, we used a volume-selective z-shim EPI technique to acquire the functional images (Du *et al*, 2007). This z-shim EPI technique can effectively reduce the susceptibility-induced signal dropout at the OFC with a minimal increase of the repetition time (TR). In this study, we acquired whole-brain functional magnetic resonance imaging (fMRI) scans with 29 slices locations using a TR of 2 s. Z-shim compensation was applied in five out of the 29 slice locations, at the region including and immediately above the OFC. Other parameters of the EPI data acquisition were: echo time = 26 ms, flip angle = 77° , FOV = 22 cm, matrix size = 64×64 , slice thickness = 4 mm without inter-slice gap. As the effective TR was 1 s in the z-shim slices, a lower flip angle of 62° was used to maximize the image signal intensity in these slices.

For a two-stage registration of the EPI images, high-resolution T1-weighted FLAIR part-head images (29 axial slices of part head, matrix = 256×192) were acquired using the same slice angles, thickness, and gap as the EPI images.

Another high-resolution full-head 3D structural image was collected in coronal plane using an inversion-recovery SPGR sequence (TI = 500 ms, flip angle = 10° , slice thickness = 1.4 mm, 256×256 matrix, 220×220 mm FOV, bandwidth = 15.6 kHz, 124 slices).

During data acquisition, a foam pillow was used for head restraint. A vitamin E capsule was placed on the right forehead as landmark. The tasks were presented using a goggle system (Resonance Technology Inc., Northridge, CA) and responses to the urge questions were recorded using a fiber-optics response pad with four response buttons. Stimulus presentation was delivered using E-Prime (for visual presentations) and Infinity Controller (for gustatory presentations).

fMRI Data Pre-Processing

Before statistical analysis, the first seven volumes of each EPI run were discarded to allow the MR signal to reach steady state. The remaining volumes in each participant's time series were motion corrected using FSL's (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) McFLIRT Version 5.0 (Motion Correction using FMRIB's Linear Image Registration Tool (Jenkinson *et al*, 2002)) and indicated that all of the participants had minimal head movement of <1 mm within a run. The two runs were concatenated for data analyses.

fMRI data analyses were carried out using FEAT (FMRI Expert Analysis Tool) Version 5.63, part of FSL using the following pre-statistics processing: non-brain tissue/skull removal using BET (Brain Extraction Tool; Smith, 2002); spatial smoothing using a Gaussian kernel of FWHM 8 mm; mean-based intensity normalization of all volumes by the same factor; highpass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma = 50.0 s). Time-series statistical analysis was carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction (Woolrich *et al*, 2001). Based on results from our pilot study, it was determined that although the sensation of liquid was immediately detected, taste identification was not possible until roughly half-way through the

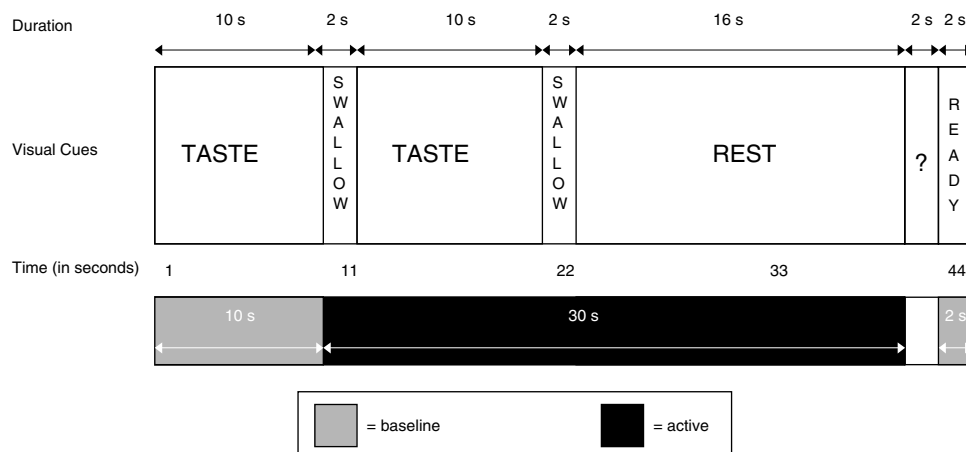


Figure 1 Schematic of a single taste cue trial. To control for taste detection and head movement, participants were asked, via visual instructions, to perform two cycles of 'taste' for 10 s with intervening 'swallow' prompts for 2 s during the 24-s taste delivery period. The taste delivery period is always immediately followed by a washout period wherein 'REST' was visually presented for 16 s. A single urge question was presented for a duration of 2 s at the end of the washout period, followed by 'Ready?' for 2 s as a prompt for the preceding the next trial. The taste and rest period of each taste cue (ie alcohol, control) are illustrated in addition to the explanatory variables (EVs) of no interest (ie urge question).

1 ml stimulus delivery, typically after the first swallow prompt. Thus, the analyses modeled the activation of the mesocorticolimbic structures after the initial swallow prompt until the end of the washout period (Figure 1).

Explanatory variables (ie taste and baseline periods for alcohol and control trials separately) were created by convolving the stimulus timing files with a double gamma hemodynamic response function in FEAT. A multiple linear regression analysis was performed to estimate the hemodynamic parameters for the different explanatory variables and a corresponding *t*-statistic indicates the significance of the activation of the stimulus. Contrast maps were created by contrasting (1) alcohol taste *vs* alcohol baseline conditions, (2) control taste *vs* control baseline conditions, and (3) alcohol taste *vs* control taste conditions. Statistical maps were then registered to the Montreal Neurological Institute (MNI) template with a two-step process. First, EPI images were registered to the part-head high resolution T1-weighted image acquired in the same plane as the EPI images. The part-head anatomical image was then registered to the high resolution full-head image, which was subsequently registered to the 152 brain average MNI template. These registration steps were performed using FLIRT (FMRIB's Linear Image Registration Tool; Jenkinson 2001; Jenkinson *et al*, 2002).

ROI Analyses

A priori region of interest (ROI) anatomical masks were created for specific mesocorticolimbic structures that have been implicated in the literature, such as the ventral striatum and dorsal striatum (VS/DS), the VTA/substantia nigra (VTA/SN), OFC, and the medial prefrontal cortex (MPFC) (David *et al*, 2005; Kalivas and Volkow, 2005; Volkow and Fowler, 2000). The VTA/SN mask was created using MRICro software (Rorden and Brett, 2000) (Figure 2) and the Tailarach and Tournoux (Tailarach and Tournoux, 1988) brain atlas was used as a guide for defining anatomical landmarks. The VS/DS, MPFC, and OFC masks were obtained from the Nielsen and Hansen's volume of interest online database (Nielsen and Hansen, 2002). After transformation of the masks into MNI space, higher-level analysis was carried out using FLAME (FMRIB's Local Analysis of Mixed Effects) (Beckmann *et al*, 2003; Woolrich *et al*, 2004). *Z* (Gaussianised *T/F*) statistic images were thresholded using GRF-theory-based maximum height thresholding with a corrected (voxel) significance threshold of $p=0.05$ (one-tailed, $Z=1.64$) (Worsley *et al*, 1992). Peak loci of activation were obtained using MRI3dX (version 5.5; <http://www.aston.ac.uk/lhs/staff/singhkd/mri3dX>) and anatomical localization was confirmed by the Talairach Daemon Database (Lancaster *et al*, 2000) and verified by the Tailarach and Tournoux brain atlas (Tailarach and Tournoux, 1988).

Whole-Brain Analyses

Exploratory analyses were carried out to investigate additional areas outside reward-craving areas that may also be involved in response to alcohol taste cues. Group analyses were carried out using a mixed effects analysis with FLAME (Beckmann *et al*, 2003; Woolrich *et al*, 2004). To control for multiple comparisons, *Z* (Gaussianised *T/F*)

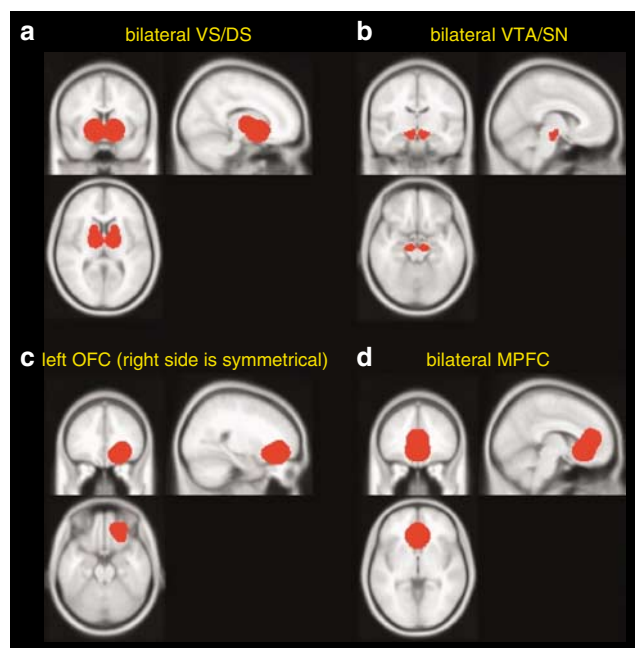


Figure 2 Regions of interest. The demarcation for the (a) right and left striatum ranged from $x: +30$ to -30 , $y: +28$ to -18 , $z: 14$ to -24 ; for the (b) right and left VTA/SN ranged from $x: +20$ to -20 , $y: -10$ to -24 , $z: -6$ to -22 ; for the (c) right and left OFC ranged from $x: +4$ to $+40$, -40 to -4 , $y: +58$ to $+14$, $z: +8$ to -24 ; for the (d) right and left VMPFC ranged from $x: -16$ to $+16$, $y: +10$ to $+54$, $z: +24$ to -24 . VS/DS=ventral striatum/dorsal striatum, VTA/SN=ventral tegmental area/substantia nigra, MPFC=medial prefrontal cortex, OFC=orbitofrontal cortex.

statistic images were thresholded at a false discovery rate (FDR) <0.05 (Genovese *et al*, 2002). For visualization and display of significant activation, the *z*-maps were overlaid on the T1 canonical MNI template using MRICro visualization software (Rorden and Brett, 2000).

Correlation Analyses

We determined functional correlation between substrates of the reward-craving pathway (ie bilateral VTA/SN, VS/DS, MPFC, OFC) by performing Pearson correlation analyses between the mean percent signal change values of these ROIs.

In order to determine the relationship between the BOLD response and behavior related to alcohol use, Pearson correlations were performed between the self-reported alcohol behavior measures (ie total AUDIT scores, AUQ baseline, and post-scan scores) and ROI maximum percent signal change values using SPSS Statistical Software *vs* 11 (www.spss.com). The maximum percent signal change per contrast for each ROIs were calculated using Featquery (part of FEAT).

RESULTS

The group of subjects' mean in-scanner urge rating for the alcohol taste cues was 1.66 ± 0.66 and for the control (ie litchi juice) taste cues was 1.5 ± 0.64 . After the scan, the subjects' AUQ scores increased by five points relative to the baseline score (ie 9.73 ± 4.77 – 14.39 ± 6.9).

A Priori ROI Analyses

To determine whether or not the control cue (juice) elicited activation in the mesocorticolimbic circuitry, the first analysis contrasted the control cue with the baseline. The contrast revealed greater activation of all of the ROIs after exposure to the juice cues (voxel-corrected $p < 0.05$, $z = 1.64$). To determine whether alcohol cues also elicited activation of these structures, exposure to alcohol was compared with baseline. Results indicated that alcohol cues resulted in significant activation within all of the ROIs (voxel-corrected $p < 0.05$, $z = 1.64$). More importantly, when alcohol cues were contrasted with the control juice cues, activity in all of the ROIs was also found to be greater during the alcohol cue (voxel-corrected $p < 0.05$, $z = 1.64$), indicating that alcohol cues produced significantly more activation than a normal, appetitive cue (Table 2 and Figure 3).

Whole-Brain Analyses

Alcohol cue vs alcohol baseline. There was widespread activity in several regions with peaks listed in Table 3. Importantly, as the regions of activation are so large in many cases, the clustering algorithm used in FSL did not always separate peaks, although there were clearly multiple peaks within large clusters. The alcohol-taste cues elicited the expected pattern of activity in the mesocorticolimbic areas, such as the VS/DS, MPFC, OFC when compared with the baseline condition (FDR-corrected $p < 0.05$). Additional areas of activity were also found in limbic cortex (insula, cingulate gyrus), parietal lobe (precuneus), the thalamus, sensorimotor cortex (pre and post-central gyrus), and occipital areas (lingual gyrus) (Table 3 and Figure 4).

Control (litchi) cue vs control (litchi) baseline. The control cues elicited a similar pattern of activity in the mesocorticolimbic areas as the alcoholic taste cues when contrasted with the baseline period (FDR-corrected $p < 0.05$). These areas included the VS/DS, MPFC, and OFC, in addition to the caudate, fusiform gyrus, precuneus, and inferior and middle occipital gyrus (Table 3).

Alcohol cue vs control (litchi) cue. The whole-brain analyses indicated that alcohol taste cues in contrast to the control (litchi) taste cues elicited greater activation in areas of the reward-craving pathway, such as the prefrontal cortex (superior, medial, middle, inferior gyrus), the cingulate gyrus and the striatum (caudate, putamen) (FDR-corrected $p < 0.05$). The parahippocampal gyrus was also activated (FDR-corrected $p < 0.05$) (Table 3 and Figure 4). Notably, areas that are not involved in the pathophysiology of alcohol dependence did not show a significant difference in their response to alcohol vs litchi. There were no areas that yielded significantly greater activation during presentation of litchi juice vs alcohol.

Correlation of Areas in the Reward Craving Pathway

Correlation analyses between the percent signal change values of ROIs showed that all except the VTA/SN mean ROI percent signal change values were highly and significantly correlated with each other (Table 4).

Table 2 Significantly Different BOLD Response in the A Priori ROI for Each Contrast

ROI	Peak	x	y	z
<i>Alcohol > rest</i>				
VS/DS	4.98	12	28	-10
	3.29	16	20	6
VTA/SN	3.68	-2	-12	-6
	3.74	4	-14	-6
MPFC	4.98	12	28	-10
	3.7	-2	52	12
	3.06	-14	42	20
L OFC	2.95	8	30	20
	4.89	-14	46	-10
R OFC	3.39	-36	38	-20
	4.98	12	28	-10
<i>Litchi > rest</i>				
VS/DS	5.71	6	-16	2
	3.16	30	14	0
VTA/SN	4.67	4	-14	-6
	4.36	-4	14	-6
MPFC	5.19	14	26	-10
	3.73	6	39	22
	2.94	-6	52	10
L OFC	4.81	-8	46	-12
	2.92	-38	26	-8
	2.57	-30	40	-16
R OFC	5.19	14	26	-10
<i>Alcohol > litchi</i>				
VS/DS	3.41	-14	-2	12
VTA/SN	2.35	-10	-16	-18
	1.8	8	-10	-14
MPFC	4.5	-6	30	14
	3.4	0	52	12
L OFC	4.13	-36	38	-20
R OFC	3.96	36	32	-18

L = left; MPFC = medial prefrontal cortex; OFC = orbitofrontal cortex; R = right; ROI = regions of interest = VTA/SN = ventral tegmental area/substantia nigra.

Local maxima for each ROI with significantly different brain activation are listed with maximum voxel Z-score, and Talairach atlas co-ordinates (TLRC) for each comparison. Listed activated brain regions had been subjected to a voxel-corrected $p < 0.05$.

Bold and Subjective Measures

There were significantly positive correlations between drinking behavior as measured by the AUDIT and ROI maximum percent signal change in the contrast of alcohol vs litchi juice for a number of regions: VS/DS ($r = 0.5$, $p = 0.002$); VTA/SN ($r = 0.38$, $p = 0.02$); R OFC ($r = 0.45$, $p = 0.006$); L OFC ($r = 0.39$, $p = 0.03$); MPFC ($r = 0.4$, $p = 0.02$) (Figure 5).

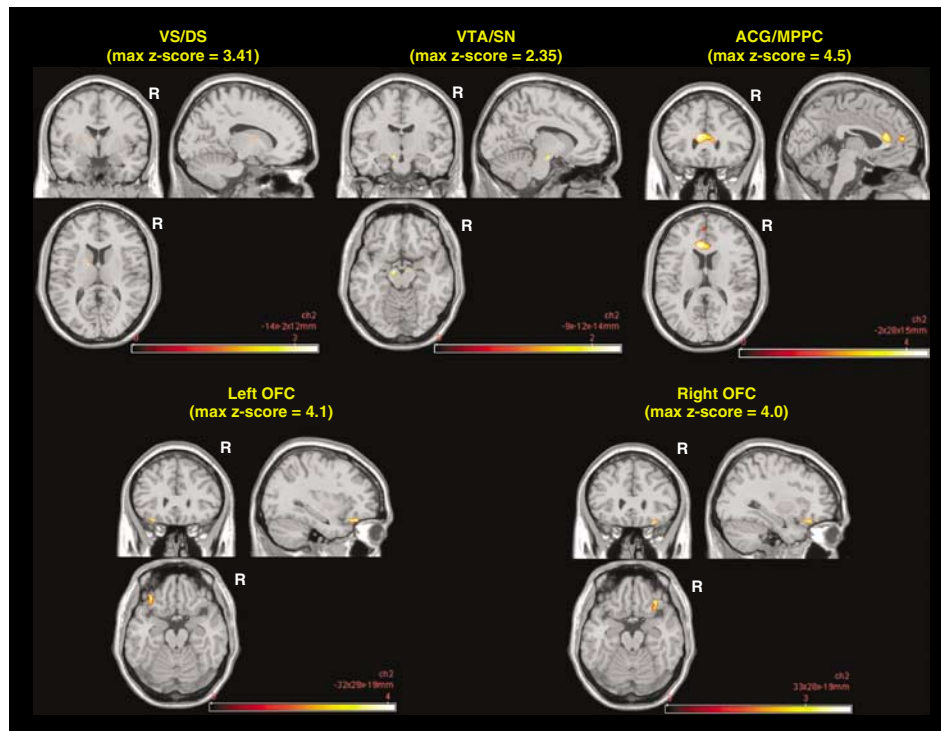


Figure 3 Greater ROI activity during alcohol vs control contrasts. All of the *a priori* ROIs showed significantly greater activity during alcohol taste cues compared with the control taste cues. Significance was determined at voxel-corrected $p < 0.05$, $z = 1.64$; Colorscale represents Z-scores; The right side of the images represent right hemispheric activations; The maximum z-values for each ROI are given; The ROIs are: VS/DS = ventral striatum/dorsal striatum; VTA/SN = ventral tegmental area/substantia nigra; ACG/MPFC = anterior cingulate gyrus/medial prefrontal cortex; OFC = orbitofrontal cortex.

There were also significant correlations between peak ROI values and subjective urge measures. The R OFC alcohol taste vs alcohol baseline contrast was significantly positive correlated with baseline AUQ scores ($r = 0.38$, $p = 0.02$) and post-scan AUQ scores ($r = 0.44$, $p = 0.007$). The MPFC alcohol taste vs alcohol baseline contrast was significantly positively correlated with baseline AUQ scores ($r = 0.33$, $p = 0.05$).

There were also significant correlations between peak ROI values and cue-induced craving scores (ie in-scanner ratings) in the R OFC ($r = 0.34$, $p = 0.05$).

DISCUSSION

The results of the present study clearly suggest that gustatory cues are a powerful stimulus for the activation of the interconnected brain structures that underlie drug seeking and motivation. The pattern of activation with gustatory cues are consistent with the pattern observed in previous studies that have relied on visual cues (Tapert *et al*, 2004; Wrase *et al*, 2002) and a combination of gustatory and visual cues (Myrick *et al*, 2004). This study represents a novel contribution to the literature, because it is the first to report that appetitive control cues (eg juice) also significantly activate mesocorticolimbic structures in heavy drinkers, and more importantly, the first to report that it is the alcohol induced increase above and beyond this activation in response to normal appetitive cues that is strongly correlated with alcohol-related problems. In addition, previous studies have not reported correlations

in the activation level between structures. The present study provides preliminary suggestions that activity between substrates in this pathway are highly correlated, suggesting a potential interconnected network that underlies urge processes (Horwitz *et al*, 2005). However, formal connectivity analyses using path analysis or correlations across the entire time series are needed to strengthen these claims.

In the present study, *a priori* ROI analyses indicated that gustatory alcohol cues elicited activation in many of the structures that have been previously implicated in the development and expression of craving for a variety of drugs of abuse including the VTA/SN (Kareken *et al*, 2004), OFC (Hermann *et al*, 2006; Myrick *et al*, 2004), and medial PFC (Kalivas and Volkow, 2005; Myrick *et al*, 2004; Park *et al*, in press). Interestingly, we did not observe differential activity in the VS corresponding to alcohol vs control cues as previously reported by other groups (eg Kareken *et al*, 2004); instead, we found increased DS/caudate activity. Increased activation for alcohol compared with the control cue was also found in the thalamus, an area that has also been implicated in cue elicited craving (Modell *et al*, 1990; George *et al*, 2001). More specifically, structures of the basal ganglia (such as the caudate) may inhibit the globus pallidus, which subsequently disinhibits the thalamus when task relevant/rewarding cues are presented, allowing the thalamus to become active (eg Frank *et al*, 2001) and subsequently activate projections to frontal cortex. The activation seen in the caudate is consistent with the proposed role of this region in instrumental responding during habit learning (Atallah *et al*, 2007) and may represent a potential activation of motor representations

Table 3 Significant Areas of Activation in Response to Taste Cues

Localization	BA	Volume	Max Z	x	y	z
<i>Alcohol > rest</i>						
L postcentral gyrus	3	55325	5.4	-26	-28	50
R superior temporal gyrus	38	449	3.1	46	10	-12
R superior temporal gyrus	22	317	3.4	68	6	-2
R middle temporal gyrus	21	77	2.8	74	-24	-4
L precuneus	7	53	2.6	-22	-58	34
R inferior frontal gyrus	45	53	2.7	52	28	6
R inferior frontal gyrus	45	26	2.7	64	30	6
L inferior frontal gyrus	45	10	2.5	-58	24	12
<i>Litchi > rest</i>						
L thalamus	—	72952	5.5	-5	-22	0
L middle temporal gyrus	21	88	2.4	-68	-40	-18
L superior temporal gyrus	29	29	2.2	-54	-40	14
<i>Alcohol > litchi</i>						
L anterior cingulate	24	5843	4.2	-6	30	14
R middle temporal lobe	21	1586	2.1	40	2	-40
L orbitofrontal gyrus	47	1436	4.1	-36	34	-20
L inferior parietal lobe	40	844	4.6	-50	-62	44
L middle frontal gyrus	6	399	3.2	-38	2	52
L posterior cingulate	29	350	3.7	-10	-48	14
L parahippocampal gyrus	34	140	3.7	-20	-12	-20
R temporal lobe	20	95	3.1	48	-18	-24
R inferior frontal gyrus	45	90	3.3	62	28	10
L lingual gyrus	30	67	3.0	-18	-50	-2
R caudate tail	—	46	3.3	24	-26	18
L thalamus	—	46	3.0	-22	-26	16
L fusiform gyrus	20	33	3.0	-40	-38	-14
L subcallosal gyrus L	25	27	3.2	0	10	-16
L culmen	—	26	3.1	-2	-38	-8
R temporal lobe	22	19	3.0	30	-40	16
L middle occipital gyrus	18	10	3.0	-44	-88	-4

L = left, R = right.

The exploratory whole-brain analyses revealed widespread patterns of activity in response to taste cues after FDR correction of $p < 0.05$ on spatially normalized (voxel size $2 \times 2 \times 2$ mm) images to the standard space of Talairach and Tournoux using the MNI template. This table reports the peaks within the broad areas of activity as determined by FSL's cluster program using a minimum cluster size of 10 voxels and connectivity radius of 26 voxels. Anatomical label, corresponding Brodmann area (BA), maximum voxel Z-score, Talairach atlas co-ordinates (TLRC), and cluster sizes are listed for significant peak areas of activity for each comparison and are listed in descending order volume, which is given as number of voxels.

involved in the seeking of drugs (Everitt and Robbins, 2005). Not surprisingly, increased activity was also observed in the insula, an area that serves to process gustatory information, particularly those with emotional valence (O'Doherty *et al*, 2001). Activation of the insula has also been implicated in the long-term effects of drugs and craving (Goldstein and Volkow, 2002) and has been shown to predict relapse (Paulus *et al*, 2005). More recently, it has

also been shown that damage to this area diminishes nicotine addiction (Naqvi *et al*, 2007). Our findings of cue-elicited activation of the fusiform gyrus (FFG) are in accord with previous findings using visual cues (Wrase *et al*, 2002).

The exploratory whole-brain analyses revealed involvement of additional areas in response to alcohol taste cues, such as widespread activity in the PFC that included dorsal PFC. As the dorsal PFC is an important area in arousal and attention, we propose that greater activity in this area during alcohol cues compared with the control cues may be due to greater attention to the alcohol cues above and beyond the control cue (Foucher *et al*, 2004). It is not surprising that activity in these areas was found as these areas have a high concentration of DAergic projections. The whole brain analyses also revealed activity in the parahippocampal gyrus after exposure to the alcohol cue (similar to Schneider *et al*, 2001; Park *et al*, in press), which has previously been associated with craving for food in human neuroimaging studies (eg Pelchat *et al*, 2004) and is likely activated because of the participants' prior learned associations with alcohol. Although whole brain analyses revealed activation in important regions in response to alcohol cues, activity in relatively small mesolimbic structures such as NAc and VTA/SN may have been missed due to the severity of the multiple comparison correction (Poldrack, 2007).

It is important to note similarities and differences between the methods and findings of the present study and the methods and findings of previously published neuroimaging studies. In the present study, the appetitive control cues activated the mesocorticolimbic circuitry as compared with a baseline. Several previous studies have not reported significant activation in the mesocorticolimbic circuitry in response to appetitive control cues (eg juice in the present study) (Myrick *et al*, 2004; Tapert *et al*, 2004; Wrase *et al*, 2002). Cues that control for normal appetitive motivation are critical in order to discriminate between activation that results from benign appetitive cues and activation that results from alcohol or drug cues. The approach in the present study was to compare the appetitive control cue to a resting baseline in order to demonstrate that the control cue resulted in expected activation of the mesocorticolimbic circuitry. The alcohol stimuli were compared with both a resting baseline, as the less stringent test, and an appetitive control stimulus, which was conceptualized as a more stringent test of the hypothesis that alcohol cues elicit hyperactivity of the circuitry. A task that allows for significant activation of the mesocorticolimbic circuitry in response to appetitive control cues allows for conclusions regarding the effect of alcohol cues beyond what one would expect from a benign appetitive stimulus.

Moreover, the results indicated significant correlations between the AUDIT score as an index of alcohol use problems and the increased activation with exposure to the alcohol cues *vs* exposure to the appetitive control. This finding corroborates previous evidence of correlations between neural activity in response to alcohol cues and future relapse (Grusser *et al*, 2004), craving responses (Heinz *et al*, 2004; Park *et al*, in press), and number of drinks consumed per month (Tapert *et al*, 2003). However, this finding extends the previous literature, as the analyses did not reveal significant correlations between the AUDIT

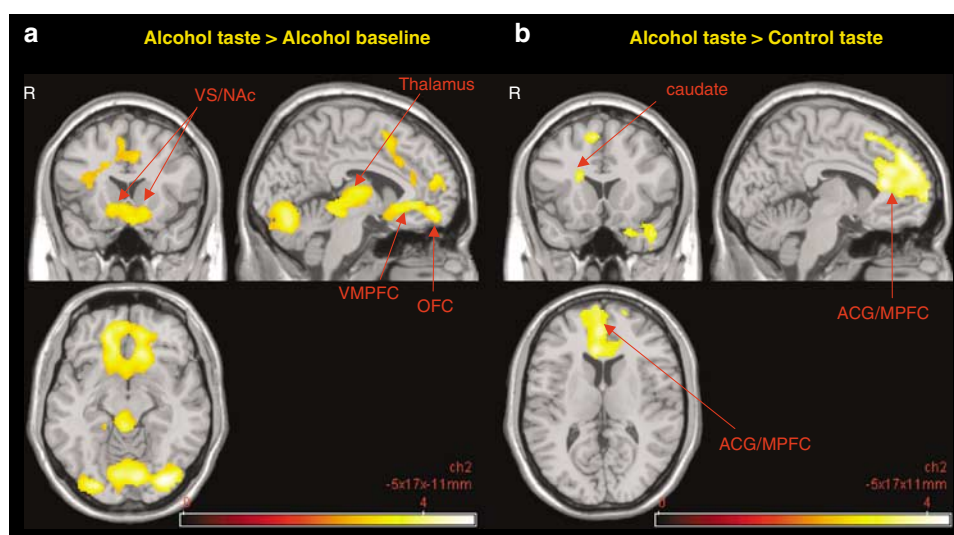


Figure 4 Areas of increased BOLD activity in response to alcohol cues as revealed by exploratory analyses. These figures illustrate widespread areas with significantly greater activity during alcohol cues compared with (a) alcohol rest and (b) control (ie litchi juice) cues during whole brain analyses. Significance was determined at FDR-corrected $p < 0.05$; Colorscale represents Z-scores.

Table 4 Correlations within the Reward-Craving Pathway Substrates

	VS/DS	VTA/SN	MPFC	Left OFC	Right OFC
<i>Alcohol vs rest correlations</i>					
VS/DS	1	0.902*	0.801*	0.778*	0.679*
VTA/SN	0.902*	1	0.613*	0.605*	0.536**
MPFC	0.801*	0.613*	1	0.896*	0.715*
Left OFC	0.778*	0.605*	0.896*	1	0.651**
Right OFC	0.679*	0.536**	0.715*	0.651**	1
<i>Alcohol vs control correlations</i>					
VS/DS	1	0.397*	0.713***	0.750***	0.838***
VTA/SN	0.397*	1	0.052	0.197	0.245
MPFC	0.713***	0.052	1	0.747***	0.802***
Left OFC	0.750***	0.197	0.747***	1	0.890***
Right OFC	0.838***	0.245	0.802***	0.890***	1

L = left, R = right, VS/DS = ventral striatum/dorsal striatum, VTA/SN = ventral tegmental area/substantia nigra, MPFC = medial prefrontal cortex, OFC = orbitofrontal cortex.

Pearson correlations between ROI mean percent signal change values are shown below.

*Correlation significant at the 0.01 level (2-tailed).

**Correlation significant at the 0.05 level (2-tailed).

***Correlation significant at the 0.001 level (2-tailed).

and the alcohol cue *vs* resting baseline comparison, suggesting that the activation above and beyond normal appetitive stimuli is the activation that is most closely associated with alcohol use problems. This finding is consistent with the theory that repeated activation of this circuitry by alcohol and drugs result in the 'hijacking' of these pathways, and the degree to which this circuitry is hijacked is associated with alcohol-related problems.

Although we expected to find correlations between changes in BOLD and changes in real-time craving scores, lack of variability in the data may have obscured this analysis. The ability to detect a difference was minimized by the decreased variability in the craving ratings due to having only four possible responses.

The overarching objective of this research project was to refine a methodology that indexes the neurobiology of craving, and thus, one that can be used to examine the effects of medications as well as genetic variation. Although mesolimbic DA projections have been clearly implicated in the attribution of incentive salience to cues after drug administration and implicated in the expression of craving, the dopamine receptor subtypes represent only one of many targets that influence this circuitry (Volkow *et al*, 2004). For example, the projections and interconnections are not solely dopaminergic, but also involve GABA, glutamate, opioid, and cannabinoid systems (reviewed by Nestler, 2004). Recent work has suggested that medications targeting the opioid system (eg naltrexone (Ameisen, 2005) and nalmefene (Anton *et al*, 2004)), the dopamine system (eg olanzapine; Hutchison *et al*, 2006), as well as GABA and glutamate (eg topiramate (Rubio *et al*, 2004) and acamprosate (Mason, 2005)) influence alcohol consumption. In addition, genetic variation in GABA function (Edenberg *et al*, 2004; Covault *et al*, 2004), opioid function (Ray and Hutchison, 2004; Oslin *et al*, 2003), dopamine function (Hutchison *et al*, 2003; Hutchison *et al*, 2006) and cannabinoid function (Zhang *et al*, 2004) have also been linked to the etiology of alcohol dependence. With a task that indexes the activation of the mesocorticolimbic circuitry, future studies may effectively combine a neuroimaging approach with tests of a specific medication or genetic variation to further elucidate basic mechanisms that may be involved in the etiology and treatment of alcohol dependence.

Although attempts have been made to control for possible confounds, we acknowledge that caution should be taken in

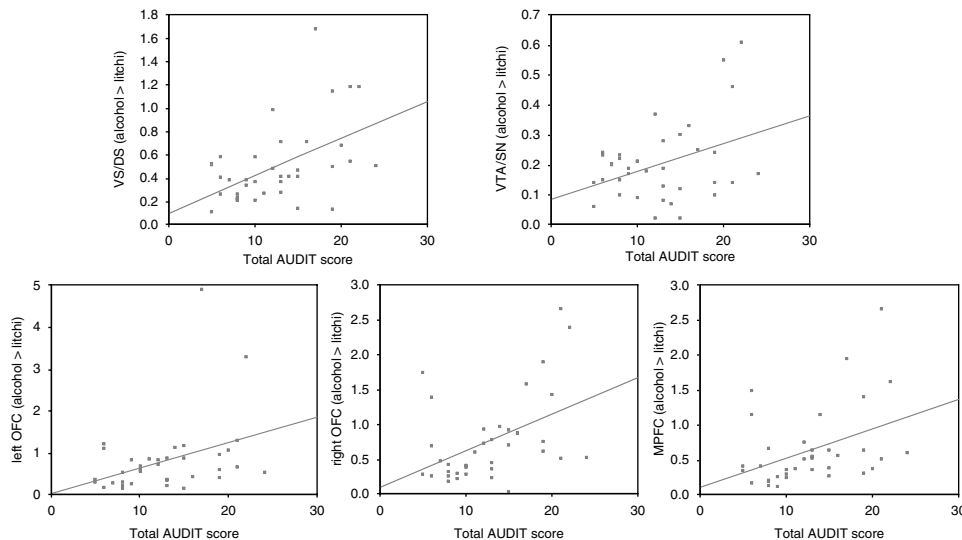


Figure 5 Correlations between ROI maximum percent signal change and drinking behavior. These plots illustrate the significant associations between brain signal in response to alcohol cues (over control cues) and drinking behavior as assessed by the total AUDIT score.

the interpretation of these findings. First, swallowing motions during the scan could have potentially introduced movement artifacts in the signal. However, we believe that any artifacts are at a very minimum because measured movement (ie rotation and translation) during each run was <1 mm for all subjects. In addition, we cannot rule out the possibility that at least some of our effects across all comparisons may be, in part, due to olfactory processing of the cues. It is well established that flavor processing involves both gustatory and olfactory contributions, and that the neural responses to these different sensory modalities overlap (eg Rolls and Baylis, 1994; Small *et al*, 1996). However, we do not believe that this confound would change our results in the reward pathway, as activity is likely due to the association of the cue (regardless of whether it is olfactory or gustatory) with the potential reward of alcohol ingestion. Lastly, it is possible that unpredicted variability was introduced in the data from selecting a non-standard alcohol taste across subjects. However, owing to known individual differences in craving, we believe that using the subjects' preferred alcoholic beverage (rather than a standard drink) actually helps minimize/controls for variances that are unrelated to the actual craving process.

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