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Alcohol Sensitizes Cerebral Responses to the Odors of Alcoholic Drinks: An fMRI Study

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Abstract

Background—Small, priming doses of alcohol enhance desire to drink, and thus play a role in the loss of control of alcohol consumption. Using functional magnetic resonance imaging (fMRI), we previously showed that alcoholic drink odors (AO; subjects' drinks of choice) induce greater nucleus accumbens (NAc) activity than non-appetitive odors (NApO; grass, leather) in subjects at risk for alcoholism. Here we hypothesized that priming exposure to alcohol would enhance responses to AO in the NAc and orbitofrontal cortex in comparison to NApO (grass, leather) and to the appetitive control odors (ApCO) of chocolate and grape.

Methods—Ten hazardous drinkers (mean age = 22.7; SD = 2.9, average drinks per drinking day = 5.9, SD = 2.3; drinking days/90 days = 50.4, SD = 13.7) were scanned on a 1.5T GE Signa MR scanner during intravenous infusion of lactated Ringer's or 6% ethanol in lactated Ringer's that was pharmacokinetically modeled to achieve a constant breath alcohol concentration (BrAC) of 50 mg % throughout imaging. During scanning, subjects sniffed AO, NApO, and ApCO.

Results—Alcohol infusion enhanced the contrast between AO and NApO in the NAc, and in orbitofrontal, medial frontal, and precuneus/posterior cingulate regions. The contrast between AO and appetitive control odors (ApCO; chocolate and grape) was similarly larger in the orbital, medial frontal, precuneus, and posterior cingulate/retrosplenial areas, with the most robust finding being a potentiated response in the posterior cingulate/retrosplenial area. The orbital region is similar to an area previously shown to manifest satiety-related decreases in activity induced by food cues.

Conclusions—The results suggest that priming exposure to alcohol renders a limbic network more responsive to alcohol cues, potentially enhancing desire to drink.

Keywords

fMRI; Olfaction; Alcohol; Alcoholism; Orbitofrontal; Nucleus Accumbens; Posterior Cingulate

THE MESOCORTICOLIMBIC DOPAMINE (DA) system plays a prominent role in addiction to alcohol and other drugs of abuse (Gonzales et al., 2004; Robinson and Berridge, 1993). While alcohol itself has been reported to increase dopaminergic transmission in the ventral tegmental area (VTA) and nucleus accumbens (NAc; e.g., Brodie et al., 1999; Di Chiara and Imperato, 1988), recent research suggests that alcohol's conditioned cues (e.g. the sight or smell of an alcoholic beverage) may be more responsible for eliciting DA activity in the NAc

(Doyon et al., 2003, 2005; Katner et al., 1996; Katner and Weiss, 1999; Melendez et al., 2002). In addition, primate and human literature point to orbitofrontal cortex's role in responding to rewards and their conditioned cues, and in a manner that varies according to reward satiety (Critchley and Rolls, 1996; O'Doherty et al., 2000; Gottfried et al., 2003).

Alcohol-related olfactory cues may be powerful appetitive cues, particularly insofar as they are present both before (nasally) and during drinking (retronasally). This would make them effective conditioned cues of alcohol's immediate reward. In support of that postulate, Grüsser et al. (2000) found that the odor of brandy (although not beer), elicited a significant craving response. Rohsenow et al. (1994, 1997) showed that combined visual and olfactory cues elicited an urge to drink in 30 male alcoholics in treatment. Weinstein et al. (1998) similarly found that combined visual and olfactory cues provoked craving and elevated systolic blood pressure in 14 abstinent male alcoholics. Using fMRI, we showed that alcohol-related olfactory stimuli elicited greater activation particularly in the NAc than non-alcohol-related odors in heavy drinkers (Kareken et al., 2004).

Priming with alcohol increases the desire to drink (De Wit and Chutuape, 1993; De Wit, 1996) and may have disinhibiting effects that lead to loss of control of drinking (Collins, 1993; Poulos et al., 1998; Bensley, 1991). This suggests the possibility that alcohol enhances sensory processing of alcohol-related cues in areas related to appetitive drive, such as the NAc and orbitofrontal cortex. While a number of authors have studied cerebral activity as a function of alcohol-associated cues (e.g., George et al., 2001; Kareken et al., 2004; Myrick et al., 2004; Tapert et al., 2004), none have done so in ways that permit studying how the pharmacologic effects of alcohol affect sensory cue processing.

We used fMRI to study regional cerebral responses to the odors of alcoholic beverages during constant, low-level brain exposure to alcohol as pharmacokinetically modeled by the "Indiana Clamp" (Ramchandani et al., 1999b; O'Connor et al., 2000; Ramchandani et al., 1999a)—a technique that permits targeting and maintaining a specific arterial blood-alcohol concentration during imaging. Ten hazardous, nondependent drinkers were exposed to the odors of their preferred alcohol drinks (alcohol odors, AO), non-appetitive odorants (NApO; grass, leather), and appetitive control odors (ApCO; chocolate, grape) under both alcohol (clamped at 50 mg %) and placebo infusions. In light of our previous findings in which the NAc responded more to AO than NApO (Kareken et al., 2004), and in the context of literature describing orbital cortex's role in reward, we hypothesized that priming exposure to alcohol would enhance the contrast between responses to alcoholic drink odors and responses to control odors in the NAc and orbitofrontal cortex.

MATERIALS AND METHODS

Subjects

Ten hazardous drinkers (4 women) who did not fit criteria for alcoholism, but who frequently drank at least 4 drinks per occasion (3 for women; NIAAA, 2007; Dawson, 2000; Dawson et al., 2005) were recruited from the community (Table 1). All voluntarily signed informed consents approved by the Institutional Review Board of the Indiana University School of Medicine. The subjects' responses on the Time Line Follow-Back Interview for drinking (Sobell et al., 1986), reflecting the 3 months immediately prior to study enrollment, showed the sample to have a mean of 21.42 (SD = 5.06) drinks per week, 5.9 (SD = 2.3) drinks per drinking day, and 1.64 (SD = 0.65) risky/hazardous drinking days per week (min = 0.6, max = 2.5). Men and women did not differ significantly in either drinking pattern or family history. Five subjects had a positive family history of alcoholism (two or more first- or second-relatives with probable alcoholism) as assessed with the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA; Bucholz et al., 1994). The sample's average score on the Alcohol Use

Disorders Identification Test (AUDIT; Saunders et al., 1993) exceeded the commonly used cutoff of 8, which is considered suggestive of alcohol-related problems (Conigrave and Hall, 1995; Conigrave and Saunders, 1995). All subjects had normal scores on the University of Pennsylvania Smell Identification Test (Doty, 1995), and all denied any Axis-I psychiatric disorders or neurological conditions of cerebral origin in a screening interview. Subjects were screened for drugs of abuse (amphetamines/methamphetamines, barbiturates, benzodiazapines, cannabinoids, cocaine, opiates, and PCP [1-phencyclohexyl piperidine]) on the day of imaging. One subject reported having smoked marijuana the night before imaging, and tested positive for cannabinoids on the day of an alcohol infusion (but not on the placebo day). The remaining subjects had negative toxicology screens.

Procedure

Alcohol Infusion—Subjects underwent fMRI on 2 different days, with an average of 27.4 days ($SD = 29.22$) between imaging sessions. Each of these sessions involved either alcohol or lactated Ringer's (placebo), in counter-balanced order across subjects. Infusion pump rates were controlled by a computer, with the infusion rate profile customized for each individual to achieve the same time course of BrAC for all subjects: A linear ascension to 50 mg% in 10 min, followed by constant exposure at 50 mg% throughout image acquisition. The placebo session infusion used the same pump-rate profile that was computed to target the BrAC of 50 mg% in the individual's alcohol session, but infused only the Ringer's lactate vehicle. Prior to and after every imaging session, breath alcohol was measured with an Draeger Alco-Sensor® IV breath alcohol meter (Intoximeters, Inc., St. Louis, MO).

While in the scanner, subjects orally rated their subjective responses to the alcohol infusion on the “high” (operationally defined in our laboratory to the subjects as, “up-stimulated, feeling good”) and “intoxicated” (“drunk, tipsy, inebriated”) items of the Subjective High Assessment Scale (SHAS; Schuckit et al., 2000). Before starting the infusion pump, all subjects were instructed to use a uniform baseline of zero (although one subject in the placebo session gave a baseline “high” rating above zero). After the infusion pump was started, the subjects' ratings of their perceived “high” and “intoxication” were allowed to range from their baseline of 0 to a maximum of 100 (the most “high” or “intoxicated” ever). Ratings of these subjective perceptions were obtained 8 times, comprising the baseline, once after reaching the calculated blood alcohol target just before beginning functional scanning, 5 times in between each of 6 imaging runs, and once after the final (6th) imaging run.

Olfactory Task—Odors were delivered using an 8-channel air-dilution olfactometer based on the design of Lorig et al. (1999), and as previously used and described in Kareken et al. (2004). Briefly, odor delivery was computer-controlled using DasyLab® software (IO-Tech, Inc., Cleveland, OH) and a Personal Daq/56 module (IO-Tech, Inc., Cleveland, OH). Air was delivered to the subject's nose via a small polytetrafluoroethylene tube at 2.0 liters per minute (lpm). This airstream was composed of a constant 1.0 lpm stream into which was injected either a second 1.0 lpm stream of clean air (between odorant stimulation), or an airstream that had been passed through one of 6 glass vials containing the odorants used in the study. In this manner, the odorants were delivered without any change in flow rate or somatosensory stimulation at the nose.

Three classes of odorants were used: (1) Alcohol Odors (AO) were each subject's two most frequently consumed alcoholic beverages, and were represented by the actual alcoholic drinks as “bubbled” (rendered volatile by passing an airstream through the liquid) in two of the olfactometer's vials, (2) Non-Appetitive Odors (NApO; Grass and Leather International Flavors & Fragrances, Union Beach, NJ) to serve as control stimulation, and (3) Appetitive Control Odors (ApCO; hot chocolate and grape juice, McCormick & Company, Inc., Hunt

Valley, MD) to serve as a second baseline reference that, like the AO, represented something comestible. Small, porous polyethylene discs (0.475 inches in diameter, 0.250 inches in thickness; Porex®, Fairburn, GA) were used to absorb the NApO and ApCO odorants, and then placed at the bottom of a glass vial, over which the olfactometer airstream passed before being delivered to the subject. After completing the imaging session, subjects left the scanner room and rated the intensity, pleasantness, and representativeness of the odors that were delivered during scanning on a linear 9-point visual analog scale.

Cue-Exposure Paradigm—Before imaging, subjects were familiarized with the odorants used during scanning by presenting the odorants in synchrony with representative images as displayed on a computer screen (e.g., beer odor presented with an appealing photograph of a frosted mug of beer). Prior to this cue-exposure and after each stimulus class (AO, NApO, ApCO), subjects answered questions from the Alcohol Urge Questionnaire (AUQ; Bohn et al., 1995) to obtain a baseline rating of desire to drink. Subjects also rated mood on the same visual-analogue scale as used for the AUQ (i.e., range 1–7) to the following prompts: “I am depressed, angry, worried or frustrated” and “I am happy, joyful or pleased.” As with the AUQ, ‘1’ represented “Strongly Disagree” and ‘7’ represented “Strongly Agree.”

Image Acquisition

All imaging was performed on a 1.5T Signa GE LX Horizon scanner (Waukesha, WI). A heavily T1-weighted, spoiled gradient echo recalled sequence (SPGR; 124 slices; voxel size, 0.9375 mm × 0.9375 mm × 1.2 mm; field of view [FOV] = 24 cm × 24 cm; repetition time [TR] = 35 ms; echo time [TE] = 8 ms; flip angle, 30°) was first acquired for anatomic registration of the functional images. Six functional image time series (126 image volumes per series) of the blood oxygen level-dependent (BOLD) response to the odorants were then acquired using a dual echo spiral pulse sequence (Li et al., 2006; Preston et al., 2004) acquisition (22 contiguous slices acquired sequentially beginning with the most inferior; voxel size, 3.75 mm × 3.75 mm × 4.00 mm; FOV = 24 cm × 24 cm; TR = 2000 ms; TE1 = 35 ms, TE2 = 70 ms; flip angle, 90°). This sequence has considerably reduced susceptibility dropout in lateral and medial prefrontal, amygdala, and medial temporal regions. The first four image volumes were discarded from analyses to account for presaturation effects. BOLD images were reconstructed by weighting the echoes according to the expected signal levels under T2* decay, such that the first echo was weighted twice as much as the second echo.

Activation Paradigm—Odors were presented in a boxcar format (Fig. 1) during three 36 second periods, alternating with periods of sham valve openings (controls) that only shunted the same non-odorized control air stream used between odorant stimulations. Within a given 252 seconds functional time series, all three 36-second odor blocks consisted of a single stimulus class (AO, NApO, or ApCO). In the first 3 functional times series acquisitions, stimulus classes were presented in a pseudo-random sequence across subjects, either (NApO, ApCO, AO), (ApCO, AO, NApO), or (AO, ApCO, NApO). For every subject, this sequence of 3 times series acquisitions was then replicated, resulting in 6 functional time series per subject/session.

Within an odorant block, four odorant stimulations were delivered in alternate order (e.g. Beer, Whiskey, Beer, Whiskey) with a 9 second stimulus onset asynchrony (SOA), during which subjects heard through noise dampening headphones, “Ready, Sniff, [Tone].” A computer-controlled odorant valve opened for a 2-second period to release the odorant at the “Sniff” command and closed at the tone, which signaled subjects to exhale. Subjects had been instructed to press 1 of 2 buttons on a response-box (Neurostim, Sterling, VA) after the tone to reflect their ability to detect the odorant (button 1 = yes, button 2 = no). This procedure served to help subjects focus on the task, and to verify compliance.

Imaging Processing and Data Analysis

Data were analyzed using SPM2 software (Wellcome Department of Imaging Neuroscience, University College, London, UK), with both placebo and alcohol session preprocessed together. Functional images volumes were slice-timed and realigned to the mean functional image volume. After co-registration to the mean functional image volume, each subject's anatomic image was normalized to stereotactic (MNI) space using the default SPM template. The normalization parameters were then applied to the previously realigned functional images volumes. Finally, the BOLD images were smoothed with an 8 mm full-width half maximum (FWHM) Gaussian kernel.

Within-subject effects were first estimated in each individual subject using a fixed-effects model in which both the placebo and alcohol sessions were modeled. Linear drifts were corrected, but temporal smoothing or autoregression was not used given the long SOA (Della-Maggiore et al., 2002). The discrete 2 second periods of odorant (or sham) valve openings (light gray tracing, Fig. 1) were convolved with SPM's canonical hemodynamic reference function, which initial analyses showed to provide better olfactory sensory system responses (activation in piriform and orbitofrontal cortex) than convolution with the entire 36-second blocks of odor valve openings (black dashed tracing, Fig. 1). A binary mask was applied to constrain within-subject analyses to gray matter by using each subject's own smoothed (8 mm FWHM) gray matter mask, as derived by SPM2's segmentation algorithm. This fixed-effects model was then used to create contrast images representing the average activation effects of AO, NApO, and, ApCO (each of these odor types compared to sniffing non-odorized air) in alcohol and placebo sessions.

Random effects analyses were used for statistical inference on a voxel-by-voxel basis (height threshold $p < 0.001$, uncorrected). Paired t tests were used to test for significant differences between the odorant classes (e.g., the hypothesis [AO > NApO]). We focused principally on differences between odorant classes, rather than the effects of an odorant class compared to an odorless baseline, so as to control for the nonspecific effects of olfactory stimulation, *per se* (Kareken et al., 2004). In addition to the voxel-by-voxel analysis, activation data for the effects of interest from *a priori* volumes of interest in the left and right nucleus accumbens (1536 mm³ volume on each side) were extracted using the Marsbar toolbox for SPM2 (Brett et al., 2002).

Due to attrition and image data corruption, 9 subjects' image data were available for analysis for the alcohol session. Image data for 8 subjects were available for the placebo session, with both sessions having the same seven subjects in common. To optimize power in this relatively small sample, we first estimated the effects of alcohol and placebo separately. However, we also directly compared the alcohol session to the placebo session using the 7-subject subset.

RESULTS

Odor Perception and Desire to Drink

Odor Ratings—Across the entire sample of 10 subjects, there were no significant mean differences in odor ratings between alcohol and placebo sessions for intensity (paired t -test, $t = 0.55$, $p = 0.58$), pleasantness ($t = 0.49$, $p = 0.62$), or representativeness ($t = 1.35$, $p = 0.18$). Ratings for alcohol and placebo sessions were, therefore, averaged for analysis. AO, NApO and ApCO were perceived as equally intense (means $\pm$ standard deviation of 7.5 ± 1.0 ; 7.1 ± 1.2 ; 7.6 ± 0.8 , respectively), although subjects perceived AO as more pleasant (6.8 ± 1.5) than NApO (5.4 ± 2.1 ; $t = 3.82$, $p = 0.0004$), but less pleasant than ApCO (8.0 ± 0.9 ; $t = -4.05$, $p = 0.0003$). The odorants were perceived as highly representative of their sources. AO (8.1 ± 1.7)

were perceived as slightly more representative than NApO (6.9 ± 1.8 ; $t = 3.44$, $p = 0.015$), but equally representative as ApCO (7.9 ± 1.1 ; $t = 0.45$, $p = 0.65$).

Desire to Drink/Mood—The effects of the odor training (odors paired with representative images) on desire to drink, as measured by the AUQ, were estimated in all 10 subjects using a 2(Session) $\times$ 4(Stimulus) within subject analysis of variance, with the Stimulus condition including the baseline and three odorant/image classes, and Session representing infusion type. There were no significant main effects for Session, or Session $\times$ Stimulus interactions. There was, however, a significant main effect for Stimulus ($F = 5.5$, $p = 0.005$). A planned comparison showed that the AUQ score after AO (and their representative images; 2.88 ± 1.67) was significantly greater than the average AUQ score over the remaining conditions (baseline, NApO and ApCO; 2.18 ± 1.07 , $F = 8.00$, $p < 0.05$). Mood ratings were not significantly different between placebo and alcohol sessions, and were therefore averaged for analysis. As assessed using two repeated measures analyses of variance, neither positive (5.20 ± 1.25) nor negative (1.90 ± 0.84) mood at baseline differed from mood as measured after exposure to NApO (positive = 5.80 ± 1.14 ; negative = 1.75 ± 0.92), ApCO (positive = 5.75 ± 1.16 ; negative = 1.85 ± 1.00), or AO (positive = 5.85 ± 1.03 ; negative = 1.80 ± 0.98).

BrAC and Subjective Effects of Alcohol—All BrACs after the placebo session were zero ($SD = 0$) by design, while the mean value of BrAC following alcohol infusion, after removing subjects from the scanner, was 50 mg% ($SD = 3.0$). Figure 2 shows perceived “high” and “intoxication” ratings as a function of each of the sequential time points at which these measurements were made. A 2(Session) $\times$ (Time) repeated measures analysis of variance, with Session representing infusion type, and Time representing the 7 time points of measurement, showed no significant main effects for Session or Time on perceived “high” (p 's > 0.5), and no significant Session $\times$ Time interaction ($p = 0.15$; all p values reported using the Greenhouse-Geisser correction for observed violations of sphericity). A similar model for perceived “intoxication” also showed no significant main effects for Session or Time (p 's > 0.5), but a borderline Session $\times$ Time interaction ($F = 3.94$, $p = 0.06$ with Greenhouse-Geisser correction). Thus, as a whole, perceived “high” and “intoxication” were highly similar across both alcohol and placebo sessions, and reflected the relatively low level of brain exposure to alcohol that was employed.

Brain Activation

Alcohol Odors Compared to Nonappetitive Odors—During placebo infusion, AO elicited no greater activation than NApO, although at a low trend threshold of $p < 0.05$, a small degree of greater AO than NApO activation was apparent in mesial frontal cortex [$-8, 56, 0$], the precuneus area [$-8, -60, 16$] (Fig. 3A), and right lateral orbitofrontal cortex [$28, 34, -10$] (Fig. 4A). Within the anatomically defined NAc ROIs (Fig. 5B; Fig. 5A for ROI definition), there was no greater activity from AO than from NApO in either the left or the right NAc (p 's > 0.24).

During alcohol infusion (Table 2), there was significantly more activation from AO than from NApO (Fig. 3B) in the NAc/olfactory tubercle area (two local maxima at [$6, 0, -10$] and [$4, 12, -8$]). There was also greater AO activation compared to NApO in medial/ventromedial frontal cortex (peak maximum at [$0, 48, -6$]), posterior cingulate/retrosplenial area (peak maximum at [$2, -42, 30$]), in the precuneus (peak maximum at [$0, -66, 24$]) and lateral orbitofrontal cortex [$34, 34, -14$] (Fig. 4B). Within the anatomically defined NAc ROI, AO provoked significantly greater activation in the left NAc than did NApO during alcohol infusion ($p = 0.05$); this comparison in the right NAc remained insignificant ($p = 0.1$).

Alcohol Odors Compared to Appetitive Control Odors—Under placebo, there was no greater activation in AO compared to ApCO, even at a trend level of $p < 0.05$. During alcohol infusion, however, AO showed greater activation than ApCO in some of the same regions that appeared in the comparison to NApO: Medial frontal cortex $[-10, 64, 10]$ and right lateral orbitofrontal cortex $[34, 36, -20]$ (Fig. 3C). We then defined 12 mm diameter spherical search regions centered on the coordinates that activated in the $[AO > NApO]_{\text{alcohol}}$ contrast and found significant posterior cingulate/retrosplenial area and precuneus activation foci (see Table 2).

There was, however, no difference between activity from AO compared to activity from ApCO during alcohol or placebo sessions in either the anatomically defined regions of NAc (Fig. 5C), or when using a spherical search area based on the NAc/olfactory tubercle region that activated in the $[AO > NApO]_{\text{alcohol}}$ contrast.

Appetitive Control Odors Compared to Nonappetitive Odors—There were no significant differences between ApCO and NApO during either placebo or alcohol.

Paired Analyses: Alcohol Compared to Placebo—To maximize power, the analyses above used all available data. However, we also conducted a secondary, but more stringent (and, because of sample size, less powerful) analysis in the smaller group of 7 subjects in whom we had both alcohol and placebo imaging data. In this case, we analyzed the $[AO > NApO]$ and $[AO > ApCO]$ difference images, submitting each to a paired- t test under alcohol and placebo conditions.

The only suprathreshold result to arise from the analysis in this smaller sample (measured either voxel-wise or in the NAc ROIs) was a potentiation of the posterior cingulate/retrosplenial region under alcohol for both the $[AO > NApO]$ $[-2, -40, 28]$, 153 voxels at $p < 0.001$ and $[AO > ApCO]$ $[6, -34, -26]$, 5 voxels at $p = 0.001$ differences images (Fig. 6). That is, the AO response (relative to either of the control odor classes) was greater during alcohol infusion than during placebo infusion.

DISCUSSION

This is the first study to use pharmacokinetically modeled intravenous alcohol infusion to study how the brain's response to conditioned alcohol cues is modified during priming exposure to alcohol. The most robust result to emerge in this sample of hazardous drinkers was the potentiation of the response to alcoholic drink odors in the posterior cingulate/retrosplenial area from alcohol priming.

Prior literature has pointed to the role of retrosplenial/posterior cingulate cortex in functions such as autobiographic memory (see Svoboda et al., 2006 for a meta-analytic review), the feeling of familiarity (Montaldi et al., 2006), emotional perception irrespective of valence (Maddock, 1999; Small et al., 2001), and perceptual decisions based on subjective preference (Johnson et al., 2005).

However, it is increasingly clear that the posterior cingulate area is part of the brain's reward circuit, and critically involved in subjective reward value (Kable and Glimcher, 2007), reward receipt (Taylor et al., 2006), and reward choice (McClure et al., 2007; McCoy and Platt, 2005). Consistent with these findings, this area has been previously identified as responding to photographs of alcoholic beverages in adolescents with alcohol use disorders, and is an area in which activity from alcohol-related stimuli correlated with the number of drinks consumed per month (Tapert et al., 2003). Hermann et al. (2006) similarly found that activity in posterior cingulate induced by the visual images of alcoholic beverages discriminated between alcoholic patients and controls.

Given Tapert's particular finding, we examined our own data *post hoc* for correlations between BOLD response and drinking patterns. While we found no correlation between the number of drinks consumed per month and BOLD signal activation in the posterior cingulate, we did observe a significant positive correlation between the number of heavy drinking days (for men, greater than 4 drinks; for women, greater than 3 drinks) and BOLD signal activation in the retrosplenial-posterior cingulate cortex (peak maximum $p = 0.002$ at $[-8, -34, 28]$; see Fig. 7), which is within 10 mm of the effect reported by Tapert et al. (2003). This positive correlation between heavy drinking days and BOLD response in the posterior cingulate reinforces the concept that this area is involved in subjective reward *value* (Kable and Glimcher, 2007). That is, individuals with a higher number of heavy drinking days presumably place a higher subjective value on alcohol and find it more rewarding. The correlation was, however, present only with the $[AO > ApCO]_{\text{alcohol}}$ contrast, and not with the $[AO > NApO]_{\text{alcohol}}$ contrast (or in any of the comparisons under placebo), which is similar to Tapert's results where the neutral condition consisted of appetitive stimuli (non-alcoholic drinks).

There was highly suggestive evidence that priming exposure to alcohol may also enhance the contrast between responses to alcohol-related odors and those of non-appetitive control odors in other areas known to be important in appetitive drive. In the largest available sample for both alcohol and placebo infusion conditions, the difference between AO and NApO was largest under alcohol in the NAc, and in medial frontal and orbitofrontal cortices, both of which project to the NAc/ventral striatum (Haber et al., 2006) and to the posterior cingulate/retrosplenial area (Kobayashi and Amaral, 2003, 2007). Activity in medial prefrontal cortex, in particular, correlates positively with subjective reward value (Kable and Glimcher, 2007), and reward receipt (Taylor et al., 2006). Similar effects were also present in comparing AO to ApCO, although in this case there were no differences in the NAc/olfactory tubercle area. Moreover, the effects were largely specific to AO, without significant differences between the two classes of control odors under either alcohol or placebo.

Orbitofrontal cortex, in particular, plays a central role in the coding of reward (e.g., Breiter et al., 1997; Kringelbach et al., 2003; O'Doherty et al., 2001), with its responses varying as a function of satiety. In primates, orbitofrontal neurons that respond to food odors decrease their responses to the odor of a food eaten to satiety (Critchley and Rolls, 1996). In humans, O'Doherty et al. (2000) and Kringelbach et al. (2003) showed that when a particular food is eaten to satiety, the perceived reward value of its odor decreases, as does the activation induced by the odor in orbitofrontal cortex; perceived reward/pleasantness and activation nevertheless remain unchanged to the odor of unconsumed food. Likewise, Gottfried et al. (2003) showed that orbital activation to a conditioned visual stimulus (an abstract design previously paired with a food odor) also varied as a function of satiety in a similar manner, as did activation in the ventral striatum, cingulate cortex, amygdala, and insula.

In the present experiment, the data suggested the inverse. That is, the relative difference between responses to AO and responses to non-alcohol-related odors was *larger* with exposure to alcohol, at least in the abusive drinkers studied here. In fact, the orbital area showing this effect ($34, 34, -14$) was highly similar to areas identified by others as showing effects related to satiety (Gottfried et al., 2003; $[24, 33, -12]$; Kringelbach et al., 2003; $[-22, 34, -8]$, although somewhat more anterior to the satiety-specific regions reported by O'Doherty et al. (2000; $[18, 18, -17]$). Similar to Gottfried et al. (2003), we also found that activation induced by the sensory properties of a reward was sensitive to reward satiety in the ventral striatum and cingulate cortex. We believe that, in these hazardous drinkers, the relatively greater response to the AO compared to control odors reflects the reinforcing effects of the priming alcohol, and increased desire to drink following that prime (De Wit, 1996; De Wit and Chutuape, 1993) particularly insofar as the targeted BrAC of 0.05 would be a relatively low concentration for subjects who habitually drink an average of 6 drinks per drinking day. While we did not directly examine

desire to drink according to stimulus exposure or infusion type during scanning, it was the case that perceived high and intoxication were not significantly different. As these subjects habitually drank to intoxication, it seems unlikely that they would have reported feeling sated by the alcohol infusion—an assumption consistent with their reported subjective effects of alcohol during imaging¹.

Thus, low-concentration alcohol exposure appears to have enhanced the contrast between responses to alcohol's conditioned cues and cues unassociated with alcohol in regions of the mesocorticolimbic pathway, at least in these hazardous drinkers. Where this effect was observed (as apparent from Fig. 5B), it was often a decrease in the NApO rather than an increase in AO (except in the precuneus and posterior cingulate/retrosplenial regions, where AO increased and NApO decreased). Such drug-related effects on the sensory properties of reward may be important to alcohol seeking. For example, Bäckström and Hyytiä (2006) found that alcohol priming significantly enhanced cue-reinstated responding for alcohol, which mirrors human data showing that low-dose alcohol exposure increases desire to drink in both social drinkers and alcoholic subjects (De Wit, 1996,2000;De Wit and Chutuape, 1993).

Frontal lobe dysfunction is thought to underlie the risk for alcoholism (Giancola and Tarter, 1999; Justus et al., 2001), with orbital dysfunction particularly suggested insofar as behavioral disinhibition and impulsiveness are risks for alcoholism (Finn et al., 1994, 2002; Finn and Hall, 2004). Dysfunction comprising the orbital system and its reciprocal targets involved in satiety and incentive salience might therefore be implicated, as well. Thus, while decreases in orbital activation as a function of food exposure may signal satiety and govern a balanced diet (Rolls, 1999), dysfunction in this system could be one factor that leads to loss of control in drinking in the early phase of alcohol consumption. Study of social drinkers who do not drink abusively, as well as alcoholic subjects, are required to test this hypothesis.

Since Kringelbach et al. (2003) and Small et al. (2001) reported correlations between orbitofrontal activation and perceived pleasantness, the greater activation caused by AO compared to NApO could be related to differences in perceived pleasantness. Two lines of reasoning suggest otherwise. First, the differential activity between these stimulus types occurred primarily in the alcohol infusion condition, whereas pleasantness ratings (made after each infusion) did not differ between alcohol and placebo infusion days. Second, the stimuli perceived as most pleasant were the ApCO. However, the AO still caused greater orbitofrontal activation than ApCO. Secondary analyses, in fact, suggest that the odors perceived as least pleasant (NApO) induced somewhat more activation during alcohol infusion in nearby orbital areas than the odors perceived as most pleasant (NApO > ApCO; 14 voxels at $p < 0.01$ at [20, 36, -20]).

There remain important limitations to consider in this study, particularly in making inferences about activity in the NAc and in medial/orbitofrontal areas. Specifically, not all subjects had available imaging data for both the alcohol and placebo sessions, with only 7 subjects having both alcohol and placebo session data. Analyses in this smaller data set also have more limited power to detect effects. While this direct paired analysis principally showed significant differences between alcohol and placebo sessions in the posterior cingulate/retrosplenial area, medial-frontal cortex continued to show significant trends suggesting that AO evoked more activity under alcohol than under placebo, mainly in contrast to ApCO (607 voxels at [-6, 34, 20] at $p < 0.05$). Orbitofrontal cortex also showed a small cluster of 27 voxels ([24, 32, -22] at $p < 0.05$) in which activity from the [AO > ApCO] comparison was greater under alcohol than under placebo. Of the 7 subjects in the smaller analyses, only 2 had a pronounced right lateral

¹In our experience, remaining supine and motionless in the scanner is another factor that renders subjects less sensitive to alcohol intoxication, likely from reduced vestibular feedback.

orbital [AO > ApCO] response under placebo, while four had a pronounced effect under alcohol (the remaining subject had a nonsignificantly greater response under alcohol). Finally, compared to our prior study (Kareken et al., 2004), we did not find NAc activation without exposure to alcohol. The reasons for this remain unclear, although one important difference between the two studies is that the prior sample of hazardous drinkers was composed uniquely of individuals with a family history of alcoholism (e.g., see Murphy et al., 2002).

We are continuing to study how olfactory cue-induced activity is modified by alcohol exposure at higher magnetic field strength, and in a larger sample of hazardous drinkers stratified by family history of alcoholism who are compared to socially drinking controls. As a whole, however, these initial results suggest that a low level of exposure to alcohol can sensitize a limbic brain network related to reward cue processing in subjects who routinely seek these rewards.

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Fig. 1.

Odor stimulation paradigm. Subjects sniffed during 2-second periods of odor stimulation (light gray tracing) across a 36-second epoch (high phase of black dashed tracing) during which the alcoholic odors (representing the subject's two most-preferred drinks), non-appetitive odors (grass/leather) or appetitive control odors (grape/chocolate) were presented. These periods were contrasted against sniffing during odorless baseline periods (low phase of the black dashed tracing). Each 36-second epoch (odor or control) consisted of four valve (odor or sham) openings (light gray tracing) following auditory commands (gray insert).

Fig. 2. Ratings of perceived “High” and “Intoxication” under alcohol and placebo infusions (**left scale**). Dashed reference line represents the modeled BrAC clamp (**right scale**). Time 1 = Baseline (immediately before the infusion pump start). Arrow = start of scanning.

Fig. 3.

(a) Trend-level activation (display threshold, $p < 0.05$) in which activity from AO was greater than activity from NApO in ventromedial frontal cortex and posterior cingulate. (b) Greater AO than NApO activation during alcohol infusion in right nucleus accumbens/olfactory tubercle area, ventromedial frontal cortex, posterior cingulate/retrosplenial cortex, and precuneus area (display threshold, $p < 0.005$). (c) Greater AO activation compared to ApCO (grape and chocolate) during alcohol infusion in ventromedial frontal cortex and posterior cingulate/retrosplenial areas (display threshold, $p < 0.005$).

Fig. 4. Orbitofrontal cortex effects as a result of AO, relative to NApO and ApCO, under placebo and alcohol infusion (display threshold, $p < 0.05$).

Fig. 5.
(a) Region of interest (ROI) analysis showing ROI volume for nucleus accumbens in stark white ($y = 8$ mm) and the average effect in this ROI for the contrasts, **(b)** AO compared to NApO; **(c)** AO compared to ApCO.

Fig. 6.

Greater activation during alcohol infusion, as compared to placebo infusion, in the $n = 7$ paired analysis (display threshold, $p < 0.005$). Significant effects for the comparisons: **(a)** [Alcohol odors > Non-Appetitive odors] (AO > NApO) in posterior cingulate and precuneus; **(b)** [Alcohol odors > Appetitive Control odors] (AO > ApCO) in posterior cingulate.

Fig. 7. Plot of the [AO > ApCO] effect under alcohol as a function of number of heavy drinking days within a 90-day period (mean-centered across the sample) in the retrosplenial cortex peak maximum $[-8, -34, 28]$. Blue arrow indicates the specific retrosplenial cluster of voxels from which the local maximum was extracted and plotted (display threshold, $p < 0.005$).

Table 1

Subject Characteristics

	Mean	SD	% (Num)
Age	22.70	2.90	
Education	14.60	1.07	
Male			60 (6)
Caucasian			100 (10)
FH+			50 (5)
Drinks/week	21.42	5.06	
Drinking days/90 days	50.0	13.7	
Heavy drinking days/90 days	25.8	9.9	
Age drinking onset	18.00	2.16	
AUDIT	9.86	2.61	
Smokers			10 (1)

n = 10. FH+ = family history positive for alcoholism.

AUDIT = Alcohol Use Disorders Identification Test.

Activation Coordinates

Table 2

	Cluster size	Voxel statistics		MNI coordinates (mm)		
		Z	p	x	y	z
AO _{placebo} ≥ NAPO _{alcohol}			no suprathreshold results			
AO _{placebo} ≥ ApCO _{alcohol}			no suprathreshold results			
AO _{alcohol} ≥ NAPO _{alcohol}						
Nucleus accumbens/olfactory tubercle	55	4.56	< 0.001	6	0	-10
		3.78	< 0.001	4	12	-8
Ventromedial frontal cortex	119	3.46	< 0.001	-4	68	-2
		3.36	0.001	-2	62	0
		3.33	0.001	8	70	2
Right precuneus	269	3.88	< 0.001	0	-68	24
Left precuneus	130	3.65	< 0.001	-44	-78	40
Posterior cingulate/retrosplenial cortex	146	3.42	< 0.001	0	-40	32
		3.34	< 0.001	-4	-50	20
Left inferior frontal cortex	86	3.54	< 0.001	0	48	-6
Right lateral orbitofrontal cortex	12	3.42	< 0.001	34	34	-14
AO _{alcohol} ≥ ApCO _{alcohol}						
Right lateral orbitofrontal cortex	30	3.44	< 0.001	34	36	-20
Ventromedial frontal cortex	27	3.36	< 0.001	-10	64	10
Posterior cingulate/retrosplenial cortex	123	2.72	0.011*	0	-40	30
Precuneus	120	2.39	0.034*	-2	-66	20

p values uncorrected, unless otherwise noted. Italicized statistics are sub-clusters.

* p-value corrected for small volume (false discovery rate) using peak maximum coordinates from the AO > NAPO analysis as the center of a 12 mm diameter sphere.