0145-6008/04/2804-0550$03.00/0
ALCOHOLISM: CLINICAL AND EXPERIMENTAL RESEARCH

Vol. 28, No. 4
April 2004

Alcohol-Related Olfactory Cues Activate the Nucleus Accumbens and Ventral Tegmental Area in High-Risk Drinkers: Preliminary Findings

David A. Kareken, Eric D. Claus, Merav Sabri, Mario Dzemidzic, Ann E. K. Kosobud, Alexander J. Radnovich, Dwight Hector, Vijay A. Ramchandani, Sean J. O'Connor, Mark Lowe, and Ting-Kai Li

Background: The mesocorticolimbic dopamine system is implicated in motivation and reward and may be involved in the development of alcoholism.

Methods: We used functional magnetic resonance imaging to study the blood oxygen level–dependent (BOLD) response to alcohol-related olfactory stimuli (AROS; odors of beer and whiskey) and non-alcohol-related olfactory stimuli (NAROS; odors of grass and leather) in 10 high-risk (HR) drinkers (average drinks per week, 19.99; SD, 6.99; all with ≥2 first- or second-degree alcoholic relatives) and 5 low-risk (LR) social drinking controls (drinks per week, 2.82; SD, 2.87; 1 subject had 1 second-degree alcoholic relative). Data were analyzed with SPM99 and random effects analysis by using regions of interest and corrected cluster statistics ($p < 0.05$) to focus on the nucleus accumbens (NAc) and ventral tegmental area (VTA).

Results: In HR subjects, there was a greater BOLD signal increase in the NAc during AROS than during clean air. BOLD signal increases during AROS were also greater in the NAc than the signal increases induced by NAROS. The AROS signal was significantly greater than the NAROS signal in a small number of voxels in the VTA. Finally, the AROS/NAROS difference signal was larger in HR drinkers in both the NAc and VTA.

Conclusions: Alcoholic olfactory cues may invoke the dopaminergic mesocorticolimbic system to a greater degree than nonalcoholic odors and could be effective tools in exploring the role of the dopamine system in susceptibility to alcoholism.

Key Words: Olfactory Cues, Nucleus Accumbens, Ventral Tegmental Area, Risk Factors.

THE MESOCORTICOLIMBIC DOPAMINE (DA) system is implicated in motivated behavior, with evidence supporting its role in the experience of reward (Wise, 1998), and in the prediction and the perception of reward and reward-related cues (Robinson and Berridge, 1993; Schultz, 2000). A variety of natural reinforcers and drugs of abuse activate this system, making it a prominent focus of addiction research.

Alcohol directly excites DA cells in the ventral tegmental area (VTA; Brodie et al., 1999), which in turn project to the nucleus accumbens (NAc). Within the NAc, extracellular DA increases are correlated with increased alcohol consumption in rats (Katner and Weiss, 2001). Even after response extinction, olfactory cues signaling alcohol's presence elicit DA release in the NAc (Katner and Weiss, 1999), indicating that the mere promise of alcohol engages this system. Furthermore, genetic differences in alcohol preference may be related to differences in the function of mesolimbic DA regions. Weiss et al. (1993) used microdialysis to show that alcohol-preferring (P) rats have a greater DA response to orally self-administered alcohol than Wistar rats. Katner et al. (1996) further showed that alcohol expectancy without consumption provoked a differentially strong DA response in P rats compared with nonselected Wistar rats. Moreover, alcohol exposure increases extracellular NAc DA in Wistar and P rats, but not in alcohol-nonpreferring rats (Smith and Weiss, 1999). Chronic alcohol ingestion may itself reduce dorsal and ventral striatal DA, potentially contributing to alcohol's addictive properties by dampening the basal activity of this system and necessitating alcohol consumption to sustain DA levels (Rothblat et al., 2001).

Alcohol-related olfactory stimuli may be powerful appetitive signals in humans, as well as in rats. This may stem, at least in part, from direct olfactory bulb projections to the

From the Departments of Neurology (DAK, EDC, MS, AEKK, AJR), Radiology (MD, ML), Medicine (VAR, T-KL), and Psychiatry (SJO), Indiana University School of Medicine, Indianapolis, Indiana; Psychology Department (EDC), Indiana University Purdue University Indianapolis, Indianapolis, Indiana; Graduate Program in Medical Neurobiology (AJR), Indiana University, Bloomington, Indiana; and Department of Psychology (DH), Indiana University, Bloomington, Indiana.

Received for publication March 3, 2003; accepted December 2, 2003.
Supported by Grant P50 AA07611-13-P42.

Reprint requests: David A. Kareken, PhD, Neuropsychology Section (RI 1773), Department of Neurology, Indiana University School of Medicine, Indianapolis, IN 46202; Fax: 317-274-1337; E-mail: dkareken@iupui.edu.

Copyright © 2004 by the Research Society on Alcoholism.

DOI: 10.1097/01.ALC.0000122764.60626.AF

Copyright © Research Society on Alcoholism. Unauthorized reproduction of this article is prohibited.

NAc and surrounding olfactory tubercle and basal forebrain area (Price, 1990). Olfactory cues are also inevitably present during drinking, making them potentially effective classically conditioned stimuli of alcohol's immediate presence. For example, Rohsenow et al. (1997) studied 30 adult male alcoholics in treatment and found that combined alcohol-related visual and olfactory cues elicited an urge to drink and smoke. Weinstein et al. (1998) found that combined alcohol-related visual and olfactory cues also evoked craving, urge to drink, and increased systolic blood pressure in 14 abstinent male alcoholics. Grüsser et al. (2000) found that the odor of brandy, but not beer, elicited a significant craving response. In an electrophysiologic study, however, Stormark et al. (1995) reported that the odor of beer increased skin conductance and heart-rate acceleration in outpatient alcoholics, but not in social drinkers.

Schneider et al. (2001) used functional magnetic resonance imaging (fMRI) to study the cerebral response to the odor of corn spirit alcohol (38% by volume) in 10 alcoholics and 10 controls. Alcohol odor induced right amygdala/hippocampus activation in the untreated alcoholics, but not in controls. After treatment, this activation disappeared. A direct statistical comparison of the groups, however, showed only a single voxel of difference in this area at a low statistical threshold of significance. Control (nonalcohol) odors were also not used. This is probably important, because we (Kareken et al., 2003) reported robust activation of this area in healthy controls by using positron emission tomography and a mix of appetitive and nonappetitive odors. Thus, in the absence of control odors, it is difficult to determine the extent to which the response reported by Schneider et al. was specific to alcohol and/or the desire to drink.

To examine the brain's functional response to olfactory cues in the context of differential risk for alcohol abuse, we studied brain activation by using fMRI and alcohol-related (AROS) and non–alcohol-related (NAROS) olfactory stimuli in 10 high-risk (HR) drinkers. Because heavy drinking and a family history of alcoholism both confer an increased risk for alcoholism (Hasin and Paykin, 1999; Hasin et al., 1997), we hypothesized that the combination of these risk factors would be associated with a differentially greater blood oxygen level–dependent (BOLD) response to AROS in the NAc and VTA in the HR drinkers. We then compared the HR sample's AROS/NAROS difference signal with the same difference signal in a reference sample of five healthy low-risk (LR) controls. The existence of such signal and group differences might then provide a potential means of identifying individuals at risk for future alcohol dependence.

METHODS

Subjects

Ten HR subjects were recruited by general advertisement (Table 1). HR subjects reported habitually drinking at least 10 drinks per week and reported having 2 or more first- or second-degree relatives with probable alcoholism. LR controls drank very little by self-report, and only one had any family history of alcoholism (one second-degree relative; Table 1). All subjects were screened with a questionnaire before imaging to rule out axis I psychiatric disorders (except for alcohol-related diagnoses) and neurological illnesses affecting brain function. None had ever been in treatment for alcohol disorders. None of the controls smoked, whereas six HR subjects reported smoking infrequently (average of 6.33 cigarettes per day; SD, 3.50 cigarettes per day). All had a normal sense of smell, as established by the University of Pennsylvania Smell Identification Test (Doty, 1995), and smokers did not differ from nonsmokers on this test ($p = 0.49$). All subjects reported drinking beer and whiskey (used for AROS). All subjects had negative breath alcohol tests (breath alcohol concentration of 0.0 by using a handheld breath alcohol meter) before imaging. All subjects provided written, informed consent before participation in the protocol, which was approved by the Institutional Review Board of the Indiana University School of Medicine.

Table 1. Subject Characteristics

Variable	High risk ($n = 10$) Mean	High risk ($n = 10$) SD	Low risk ($n = 5$) Mean	Low risk ($n = 5$) SD
Age	22.70	3.34	27.00	5.66
Drinks/week[a]	19.99	6.99	2.82	2.87
Average drinks/day[a]	5.68	1.17	—	—
Peak drinks/day[a]	15.30	6.52	—	—
Drinking days in 90 days[a]	47.00	16.66	—	—
AUDIT	14.20	3.16	—	—
Number of FH^+ relatives	3.30	1.16	—[b]	—[b]

[a] For high-risk subjects, estimated from the timeline follow-back interview over 90 days; for controls, estimated from self-report.
[b] One control reported a single second-degree relative with alcoholism.
AUDIT, Alcohol Use Disorders Identification Test; FH, family history.

Assessment

For HR subjects, recent drinking was quantified by using the timeline follow-back (Sobell et al., 1986) method for the previous 90 days. Family history of alcoholism was determined by using the Family History Assessment Module of the Semi-Structured Assessment for the Genetics of Alcoholism (Bucholz et al., 1994). Hazardous drinking was characterized with the Alcohol Use Disorders Identification Test (Saunders et al., 1993). These instruments were completed during a screening visit.

Procedures

Image Acquisition. Functional images were acquired by using the gradient-echo echo-planar imaging (EPI)-BOLD method on a 1.5-T Signa GE LX Horizon scanner (Waukesha, WI). Functional imaging sessions comprised 160 images acquired during each of four 6-min sessions (15 slices: voxel size, $3.75 \times 3.75 \times 9$ mm; no interslice gap; repetition time (TR)/echo time (TE), 2.25 sec and 50 msec, respectively; flip angle (FA), 90°; field of view, 24×24 cm; matrix, 64×64). High-resolution, heavily T1-weighted anatomical images were acquired for anatomical comparison (124 contiguous axial slices; 3-dimensional spoiled gradient recalled acquisition (SPGR); slice thickness, 1.2 or 1.3 mm; TR/TE, 35 and 8 msec, respectively; FA, 30°; field of view, 24×24 cm; matrix, 256×128). Subjects were fitted to a bite bar to reduce motion, and headphones were used to enable the delivery of auditory commands.

Olfactory Stimulation and Imaging Task. Olfactory stimulation used an air-dilution olfactometer based on the design of Lorig et al. (1999), which was computer-controlled by using a laptop computer, Dasylab® software (IOTech, Inc., Cleveland, OH), and a Personal Daq universal serial bus data-acquisition module (IOTech, Inc.) for precise timing of the olfactometer's solenoid valves. All electronic and ferrous material was located outside the scanning room. The olfactometer was triggered electronically when the EPI imaging sequence was initiated, after which stimulus delivery and timing were controlled internally by the Dasylab software. The

Copyright © Research Society on Alcoholism. Unauthorized reproduction of this article is prohibited.

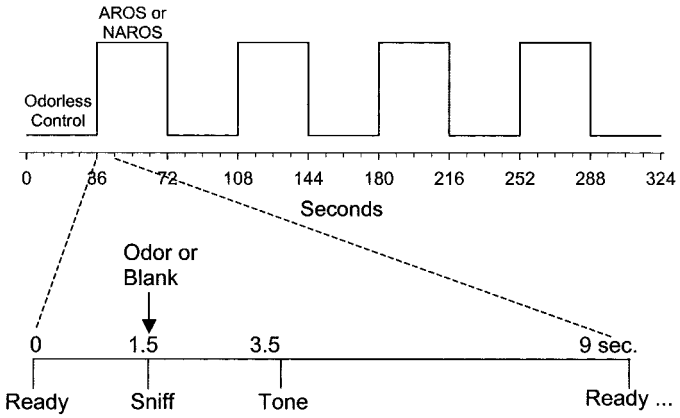

Fig. 1. Subjects were exposed to either non-alcohol-related olfactory stimuli (NAROS) or alcohol-related olfactory stimuli (AROS) during the high phase of the boxcar reference function and to odorless air during the low phase. Each session lasted 6 min, with a 9-sec odor onset asynchrony and auditory cues as depicted.

odors used in scanning were those of whiskey (Jack Daniels®, Jack Daniel Distillery, Lynchburg, TN) and beer (Budweiser®, Anheuser-Busch Inc., St. Louis, MO), whereas control odors were grass and leather (International Flavors & Fragrances, Union Beach, NJ). Grass was dissolved in odorless propylene glycol (1% concentration), and leather and the alcohol odors were undiluted. Fresh odorants were used for each subject. A small polytetrafluoroethylene nasal cannula was used to deliver the odorants birhinally.

Immediately before imaging, all subjects were trained to identify the odors by pairing each with a representative photograph displayed on a computer monitor. The Alcohol Urge Questionnaire (AUQ; Bohn et al., 1995) was administered just before and after exposure to AROS stimuli in this procedure for all HR subjects and three of the controls. During imaging, olfactory stimuli were delivered according to a boxcar reference function (i.e., a blocked design; Fig. 1) by using five 36-sec control periods of nonodorous control stimulation and four 36-sec periods of either AROS or NAROS stimulation in a given scanning session (two AROS scanning sessions and two NAROS scanning sessions in alternating order within subjects, and administered in counterbalanced order across subjects). Each olfactory or control stimulus was administered in a 9-sec time bin, during which subjects heard the aural command "Ready, sniff [tone]" through noise-dampening headphones, where the tone signaled subjects to exhale (Fig. 1). Four odorous stimuli were delivered in a given 36-sec block, and the odors in the stimulus class were alternated (e.g., beer, whiskey, beer, whiskey). To help ensure that subjects were awake, alert, and complying with the task, they were instructed to press one of two buttons on a response box (Neurostim, Sterling, VA) to indicate their ability (button 1) or inability (button 2) to smell a stimulus (odor or odorless blank) upon each delivery. All subjects performed this detection task continuously throughout scanning, in both odorant (AROS and NAROS) and odorless conditions. Detection accuracies during AROS (87%; SD, 8%) and NAROS (84%; SD, 9%) were not significantly different ($p > 0.50$) across subjects. No other questions were asked about the stimuli during imaging. Subjects were told in advance of each session which class of odors they would experience. Subjects were also instructed to keep their eyes closed during the functional scanning. After imaging, all HR subjects and three of the controls rated the intensity and pleasantness of all odors on a nine-point Likert scale (1, weak or very pleasant; 9, strong or very unpleasant) during exposure to each odor as delivered through the olfactometer outside the scanner. On a similar nine-point scale, subjects also rated the degree to which each odor smelled like the object it was intended to represent (e.g., how much did the beer odor smell like real beer).

Image Processing and Data Analysis. SPM99 (Wellcome Department of Cognitive Neurology, University College, London, UK) was used for data analysis. All raw functional scans were Hamming-filtered by using an 8.4^{-1} cm per cycle Hamming window spatial smoothing filter to improve the signal-to-noise ratio during image reconstruction (Lowe and Sorenson, 1997). Because motion analysis (Woods et al., 1992) showed maximal peak-to-peak displacements of less than 0.2 mm from use of the bite bar, no motion correction was applied. High-resolution three-dimensional SPGR anatomical images of each subject were used to derive the parameters used for nonlinear warping ($7 \times 8 \times 7$ basis functions; 12 iterations) of the subjects' images into stereotactic (Montreal Neurological Institute (MNI)) space. These parameters were then used to transform the EPI images into the same coordinate space with an isotropic voxel size of 2 mm. Final estimated image smoothness (full width half maximum) was approximately 12, 12, and 14 mm in the x, y, and z dimensions, respectively.

A random effects analytical strategy was used by first using an fMRI model in SPM99 to summarize each subject's data into a single summary contrast image of a given condition by convolving the images with a standard boxcar reference function. The initial 11 volumes were discarded to account for presaturation and the hemodynamic delay (Bandettini et al., 1993). Because statistical inference was derived from a random effects model, temporal smoothing and autoregression were not used. The contrast images were then analyzed with basic model t tests to assess the effect of a given condition. For within-group analyses of a condition effect, a one-sample t test was used to test a given effect (set of contrast images) against a null hypothesis of 0. This consisted of images whose voxels reflected [AROS > clean air], [NAROS > clean air], and [(AROS > clean air) > (NAROS > clean air)]. For group comparisons, an independent two-sample t test was used to compare contrast images across groups. Cluster statistics ($p < 0.05$, corrected) were used at height thresholds of either $p < 0.05$ or $p < 0.01$, uncorrected. The lower threshold ($p < 0.05$) allowed for a smaller signal height when comparing the two activated conditions (i.e., AROS and NAROS).

Small-Volume Correction. Because we had directed hypotheses, small-volume corrections were applied within SPM99 using the small-volume correction utility to reduce the multiple (voxelwise) comparison correction penalty. For NAc, two small ($8 \times 14 \times 8$ mm) a priori volumes of interest were applied to each NAc in the AROS condition, centered on the

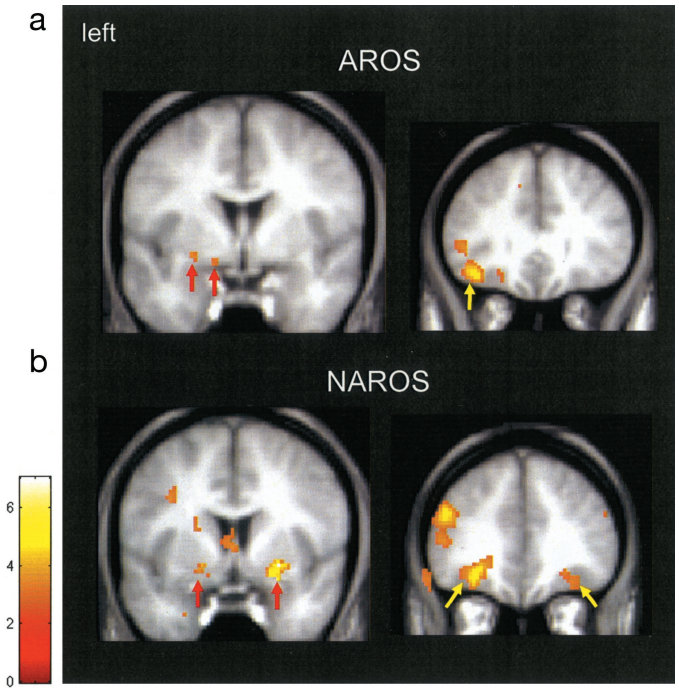

Fig. 2. Olfactory cortical system activation across all subjects from alcohol-related (AROS) and non–alcohol-related (NAROS) olfactory stimuli ($p < 0.005$; uncorrected). Red arrows, frontal piriform cortex; yellow arrows, orbitofrontal cortex; (a) coronal slices, 0 mm (left) and +32 mm (right); (b) coronal slices, +4 mm (left) and + 38 mm (right).

Copyright © Research Society on Alcoholism. Unauthorized reproduction of this article is prohibited.

Table 2. Volume of Interest Analyses for the Nucleus Accumbens and the Ventral Tegmental Area

Variable	MNI coordinates			Region	Peak height[a]	Cluster size	Cluster probability[b]
	x	*y*	*z*				
HR AROS							
	−14	4	−8	L-NAc	<0.001	67	0.001
	12	12	−6	R-NAc	0.003	54	0.003
HR AROS > NAROS							
	−10	4	−6	L-NAc	0.02	13	0.02
	14	10	−4	R-NAc	0.02	16	0.01
	6	−10	−12	L-VTA	0.02	3	0.002
LR AROS							
	−14	10	−6	L-NAc	0.002	50	0.02
HR > LR, AROS > NAROS							
	14	12	−4	R-NAc	0.02	27	<0.001
	6	−12	−12	R-VTA	0.005	13	<0.001

For LR AROS > NAROS, there were no significant clusters in volumes of interest.

Negative coordinates are left.

R, right; L, left; NAc, nucleus accumbens; VTA, ventral tegmental area; HR, high-risk subjects; LR, low-risk subjects; AROS, alcohol-related olfactory stimuli; NAROS, non–alcohol-related olfactory stimuli.

[a] Uncorrected.

[b] Corrected for search volume at heights of $p < 0.01$ uncorrected (HR AROS) and $p < 0.05$ uncorrected (HR AROS > NAROS; LR AROS; HR > LR, AROS > NAROS).

coordinate (10, 8, and −8) on each side. These locations were derived from the high-resolution Talairach-transformed anatomy of Mai et al. (1997). This permitted calculation of the corrected significance of AROS in this area alone. To compare AROS with NAROS scans, binary image masks were then made of the AROS effect (threshold $p < 0.01$, uncorrected), and only voxels within this area were analyzed (i.e., a functionally defined region of interest, based on activation of AROS). For the VTA, a small region of interest was drawn on MRI-based anatomy of the VTA region, spanning axial slices −9 mm to −15 mm in the *z* axis and corresponding approximately to the VTA as depicted in Duvernoy (1995)—i.e., the mesial-ventral midbrain roughly between the level of the mamillary bodies and just superior to the pons (Fig. 4). This 520-mm^3 region was then converted to a binary image mask, and only regions within this mask were submitted for voxel-by-voxel analysis in all of the analyses.

RESULTS

Odor Perception and Desire to Drink

All subjects (HR and LR) perceived AROS and NAROS as equally intense (paired *t* test; $p > 0.20$), although they perceived NAROS as slightly more pleasant (NAROS, 4.15; SD, 1.48; AROS = 5.46; SD, 1.22; $p < 0.005$). Smoking was not related to perception of odorant intensity or pleasantness ($p > 0.60$). The subjects also thought the odors to be reasonably representative of their source (beer, 6.85; SD, 2.12; whiskey, 6.92; SD, 1.38; grass, 7.54; SD, 1.76; leather, 5.69; SD, 1.75). Collapsing across stimulus class, the subjects did not perceive alcohol and nonalcohol odor classes to be significantly different in representativeness (AROS, 6.88; SD, 1.26; NAROS, 6.62; SD, 1.46; paired *t* test; $p > 0.60$). Before imaging, the subjects' responses on the AUQ also indicated greater desire to drink after exposure to the combined alcohol-related olfactory and visual cues during training. This was true in all subjects [paired $t(12) = -2.25$; $p < 0.05$], as well as in the HR subjects when analyzed separately [paired $t(9) = -2.26$; $p < 0.05$]. Of the three LR controls for whom we had AUQ data, their posttraining AUQ responses fell into the lowest quartile of the HR group.

Olfactory Activation

To ensure that the stimuli were first successful in activating the broader olfactory network, AROS and NAROS scans were each analyzed for activation of olfactory cortical areas in all subjects (threshold $p < 0.005$, uncorrected; Fig. 2). These analyses indicated that both AROS and NAROS sessions activated piriform cortex, the insula, and the lateral orbitofrontal cortex (for examples, see Dade et al., 2002; Kareken et al., 2001, 2003; Poellinger et al., 2001; Sobel et al., 2000; Zatorre et al., 1992). Analyses similarly indicated olfactory system activation from AROS and NAROS within each group (i.e., HR, LR) separately (fixed effects; $p < 0.05$ corrected for the whole brain, most of which was orbitofrontal). A direct comparison between AROS and NAROS contrast images did not show significant, systematic differences in olfactory cortical areas. Thus, odor stimulation invoked the olfactory system as a whole, verifying that the stimulation protocol succeeded in targeting the desired sensory network.

HR Subjects: AROS Effects

NAc. Within the bilateral NAc search volumes, there were significant increases in BOLD signals related to AROS versus clean air (Table 2; Fig. 3). These regions of activation were then turned into a binary image mask (volume of interest demarcated by activation) within the boundaries of the left and right NAc. Within this search area, significantly greater clusters of AROS signal were found in comparison to the NAROS signal, again on each side (Table 2; Fig. 3).

VTA. For the VTA, small, insignificant clusters of activity emerged in AROS alone (i.e., AROS versus clean air),

Copyright © Research Society on Alcoholism. Unauthorized reproduction of this article is prohibited.

even when constrained by the anatomical search area. When AROS was compared with NAROS, there were a very small number of voxels in the VTA mask on the left (Table 2; Fig. 4).

LR Subjects: Activation Effects

In the LR controls, AROS activated the left NAc (Table 1); a small, insignificant cluster was present in the right NAc. No significant signal was present in the VTA. NAROS did not significantly activate either the left or right NAc, although a small, insignificant cluster was found in the right NAc volume of interest. There were no significant clusters of difference between AROS and NAROS in the NAc or the VTA.

Comparison With Controls

For NAc, contrast images reflecting the AROS > NAROS effect (dubbed AROS') were compared across HR and LR groups by using the area of AROS' activation from the HR group. In this comparison, HR subjects had a significant cluster of AROS' signal in the right NAc when compared to LR subjects (Table 2; Fig. 3). Neither group was significantly greater than the other in the left NAc. A group difference was also apparent in the VTA area (Table 2; Fig. 4), the cluster size of which was significant after constraining the search to the VTA anatomical mask. Thus, HR subjects had significantly more AROS' signal than LR subjects in both the VTA and the NAc (Table 2; Fig. 4).

DISCUSSION

Compared with nonalcoholic cues, alcoholic drink odors elicited greater NAc activity in a sample of 10 HR drinkers. A similar effect was present in VTA, although the spatial extent was quite small. These findings are nevertheless consistent with substantial evidence implicating the VTA and NAc in the actions of numerous drugs of abuse in animals (Weiss and Porrino, 2002) and humans (e.g., Braus et al., 2001; Breiter and Rosen, 1999; Hutchison et al., 2001; Sell et al., 1999). Our primary intent was to establish a meaningful activation effect in a population at risk for alcoholism, and in that respect, our reference sample of LR controls was small. The interpretation of the observed group differences in activation must therefore be limited to the intriguing possibility that differential NAc and VTA responses to alcoholic drink odors might be observed in humans as a correlate of risk for alcoholism. Whether this is actually true awaits future study. These preliminary data nevertheless support the continued study of olfactory cues and their effects in brain reward areas.

Our findings also reinforce the hypothesis that these brain regions do not need a direct reward to be activated, but only cues normally associated with reward availability (Robinson and Berridge, 1993). Thus, it is possible that our odor training paradigm might have had a role in differentially activating the HR and LR groups. In this case, we might not be able to attribute all of the activating effects to the odorants themselves, because the training paradigm (which increased the desire to drink) may have differentially sensitized the HR subjects. It has also been shown that cues need not be appetitive (West et al., 1992) or even particularly reinforcing to elicit NAc DA activity (Young et al., 1998). This point was highlighted in our experiment by the fact that, although the beer and whiskey odors elicited a strong NAc response, grass and leather odors also elicited activity in the same region. Nevertheless, the NAc was differentially responsive to our appetitive stimuli. In animals, West et al. (1992) similarly found that NAc cells clearly responded to neutral and conditioned sexually appetitive odors, although the number of units responding to sexually conditioned odors was significantly greater.

Other human functional neuroimaging studies have also demonstrated activation in the NAc, either with direct reward or with cues suggesting a reward's availability. In cocaine-dependent subjects, Breiter and Rosen (1999) showed that cocaine infusion elicited an NAc BOLD fMRI response, as did cocaine expectancy without drug delivery during rescanning. These same authors also found that a nonrewarded but difficult continuous performance (work-

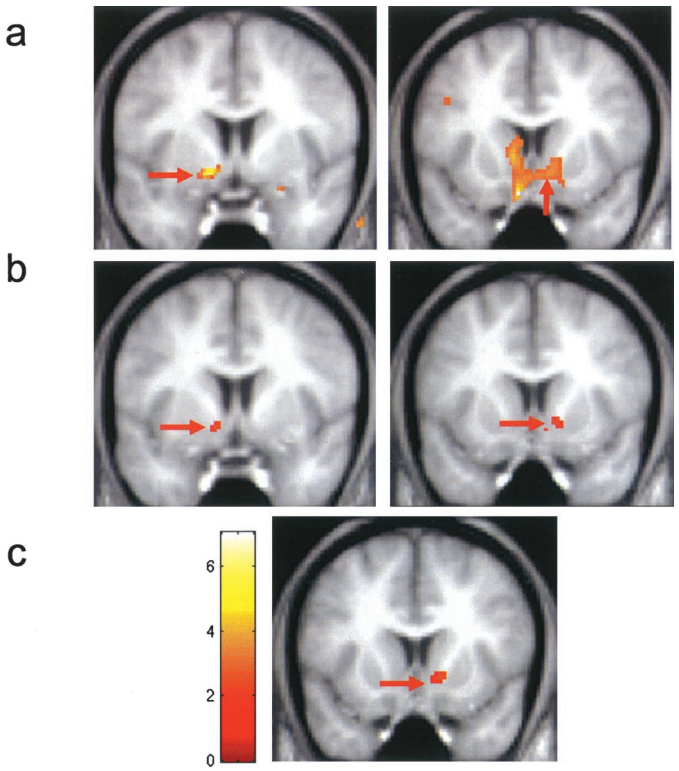

Fig. 3. (a) Activation effect of alcohol-related olfactory stimuli (AROS) in high-risk (HR) subjects. Arrows indicate the region of the nucleus accumbens (NAc; coronal slices: +4 mm left and +12 mm right). (b) There was a greater AROS than non–alcohol-related olfactory stimuli (NAROS) signal in HR subjects within a small volume of interest defined by the AROS activation in panel (a) (coronal slices: +4 mm left and +10 mm right). (c) The right NAc area had a greater signal in HR subjects compared to low-risk subjects, as masked by the activation results in panel (b) (coronal slice, +12 mm).

Copyright © Research Society on Alcoholism. Unauthorized reproduction of this article is prohibited.

ing memory) task in healthy subjects activated the NAc when compared with a simpler version of the task, placing the specificity of this system to reward in doubt. At the same time, however, it also possible that success at a difficult task is internally rewarding. Breiter et al. (2001) found that both the expectancy and experience of monetary reward in healthy subjects activated the NAc, as well as the VTA, orbitofrontal cortex, and the sublenticular extended amygdala. Knutson et al. (2001) similarly reported that anticipation of financial reward recruited the NAc. Further, the NAc signal changed in proportion to the degree of reward and correlated with self-reported happiness from the reward cues. O'Doherty et al. (2002) used fMRI to show that the anticipation of a pleasant taste reward compared with anticipation of a neutral taste activated the dopaminergic midbrain, as did anticipation of the pleasant taste when compared with the actual taste. These authors also found that the NAc was more active when a pleasant taste was anticipated than during the taste itself. Predictability of reward may also be an important factor, as unpredictable pleasant taste rewards seem to activate the medial orbital cortex/NAc area more than predictable rewards (Berns et al., 2001).

The imaging literature in alcohol abuse and alcoholism is somewhat diverse in its findings. Schneider et al. (2001) conducted a study similar to ours, using fMRI to scan 10 alcoholic subjects during exposure to a corn alcohol odor before and after detoxification. The alcohol odor did not activate the NAc or VTA regions in patients before detoxification. Instead, the authors reported a small cluster of three voxels in the right amygdala at a low statistical threshold, but they did not find any signal in this area in controls or in the same patients after treatment. However, we also reported that this same coordinate responded much more robustly to a mix of appetitive and nonappetitive odors during positron emission tomography (Kareken et al., 2003), which is logical given the direct olfactory bulb projections to this area (Price, 1990). Moreover, the Schneider et al. study did not seem to demonstrate the expected olfactory-related signals in the orbitofrontal cortex and only inconsistently showed activation in the insula. Thus, one possibility is that the alcohol odor was too weak to activate the larger olfactory sensory system, making detection of group differences in sensitivity to this odor difficult. Schneider et al. also used corn ethanol in their study, whereas our odors were those of common alcoholic beverages, paired with the images of the beverages just before scanning. Thus, both the naturalistic qualities of the odors and our explicit pairing of these odors with the enticing and representative pictures may have caused greater responsiveness in dopaminergic reward areas. Finally, the specificity of activation in the Schneider et al. study is uncertain in the absence of nonalcohol control odors.

George et al. (2001) used fMRI to scan non–treatment-seeking alcoholic patients and controls while they viewed pictures of alcoholic beverages and neutral photographs. They reported increased activity in the left dorsolateral prefrontal cortex and the anterior thalamus in the patients, but not in the controls. One possibility for the discrepancy between this study and our findings is that the olfactory system projects more directly to areas in the dopaminergic basal forebrain and that odorous stimuli are potentially more potent cues of the availability of reward than visual cues. Braus et al. (2001) used fMRI to image the effects of alcohol-associated, neutral, and abstract pictures in 4 abstinent alcoholic patients and 10 controls. They reported

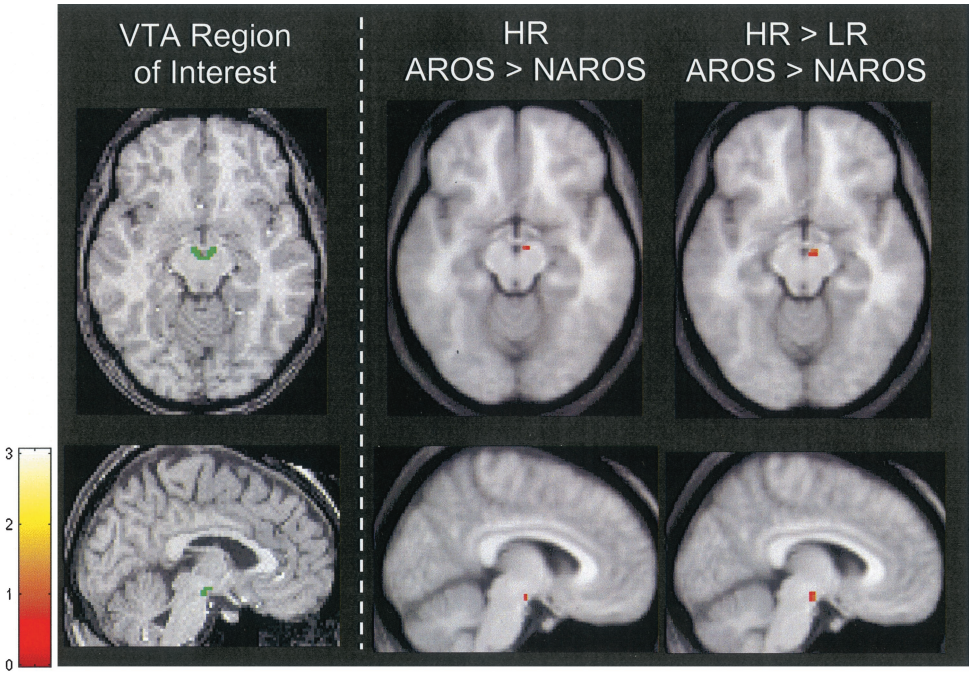

Fig. 4. (Left) Anatomical volume of interest used to delimit the ventral tegmental area (VTA; left). (Middle) Greater alcohol-related olfactory stimuli (AROS) signal than non–alcohol-related olfactory stimuli (NAROS) signal in high-risk (HR) subjects within the VTA mask. (Right) Greater AROS signal (contrasted against NAROS) in HR subjects when compared to low-risk (LR) subjects. Axial (top) slices at −12 mm; sagittal (bottom) slices at +8 mm.

Copyright © Research Society on Alcoholism. Unauthorized reproduction of this article is prohibited.

ventral striatal activation to the alcohol-related cues in the patients, but not in the controls. Wrase et al. (2002) also reported basal ganglia and orbitofrontal activation in six abstinent alcoholics who viewed enticing photographs of alcoholic beverages. Most recently, Tapert et al. (2003) studied teenagers with alcohol use disorders by using fMRI during exposure to visual cues. These authors found that, compared with age-matched healthy controls, teens with alcohol use disorders showed greater cue-related activity in limbic regions such as the amygdala and orbital cortex.

Varied findings from studies with a wide range of subject populations inhibit a consistent interpretation of the findings. Our own study used nonalcoholic heavy drinkers with positive family histories who were largely functional in their social roles and in whom drinking was more intermittent than in most alcoholics. It is conceivable that abstinent alcoholic subjects who have undergone treatment may respond differently than our HR drinkers or non–treatment-seeking alcoholics, particularly because they may have been taught to cognitively manage their reactions to alcohol-related stimuli. Within-group variability in striatal dopaminergic function itself could even potentially affect which subjects are most likely to show a response (Guardia et al., 2000). Technical factors in fMRI, such as sensitivity to basal forebrain areas from susceptibility artifacts, are also bound to contribute to the variability of the results.

There are limitations to this study. The peak height of the signals was relatively low, and how well the findings generalize to a larger sample or to other populations of interest remains to be explored in future research. The areas studied are also subject to susceptibility artifacts, and future study would benefit from pulse sequences with greater sensitivity to these areas. We also had limited data in the LR sample, which did not have strict timeline follow-back estimates of recent drinking. However, our experience in recruiting suggests that self-reported recent drinking history is a reasonably reliable estimate for this purpose. On a related note, the small control sample did not permit separate group analyses of the stimulus qualities. As a whole, there were no significant differences across stimuli in intensity. A small difference emerged in pleasantness, albeit in a direction that would have theoretically operated in the opposite direction than that observed in reward-related regions (i.e., with AROS being slightly less pleasant than NAROS). Although group differences in perceived odor characteristics might be expected, it would be best to ensure within-group equivalence in stimulus intensity in both samples. Inspection of the data did not, however, suggest any obvious stimulus intensity differences in the controls. Our subjects were also selected so as to represent the highest and lowest combination of two risk factors for developing alcoholism (drinking and family history). Thus, we cannot determine the extent to which each of these factors contributed to our findings. Finally, the findings of this experiment are not necessarily related to abusive or addictive behavior. For example, the same findings might be observed in nonaddicted subjects exposed to cues of their favorite nonalcoholic beverages. Teasing apart these more subtle effects may instead require determining whether treatment relapse occurs on the basis of function in mesocorticolimbic dopaminergic areas or whether the response of these regions predicts the development of alcoholism in subjects at risk.

In summary, olfactory cues of alcoholic beverages seem to activate dopaminergic areas implicated in alcoholism and, more generally, in the expectation of reward. Given its relatively direct projections to the basal forebrain area, the olfactory pathway may be a particularly effective route through which to elicit activity in dopaminergic regions. This study also provides a link to the animal literature on brain systems and reward, in which olfactory cues are commonly used. More importantly, the findings support the use of this approach in future studies to determine how activity in this system might predict the development of alcoholism and its relapse after treatment.

ACKNOWLEDGMENTS

We thank Stephen Warrenburg of International Flavors and Fragrances for odorants; Tracey Osborn and Jacqueline Zimmerman for technical assistance during imaging; David Crabb, Dena Davidson, Janice Froehlich, William McBride, and three anonymous reviewers for helpful comments; and Joseph E. Steinmetz for his generous donation of resources in constructing the olfactometer.

REFERENCES

Bandettini PA, Jesmanowicz A, Wong EC, Hyde JS (1993) Processing strategies for time-course data sets in functional MRI of the human brain. Magn Reson Med 30:161–173.

Berns GS, McClure SM, Pagnoni G, Montague PR (2001) Predictability modulates human brain response to reward. J Neurosci 21:2793–2798.

Bohn MJ, Krahn DD, Staehler BA (1995) Development and initial validation of a measure of drinking urges in abstinent alcoholics. Alcohol Clin Exp Res 19:600–606.

Braus DF, Wrase J, Grusser S, Hermann D, Ruf M, Flor H, Mann K, Heinz A (2001) Alcohol-associated stimuli activate the ventral striatum in abstinent alcoholics. J Neural Transm 108:887–894.

Breiter HC, Aharon I, Kahneman D, Dale A, Shizgal P (2001) Functional imaging of neural responses to expectancy and experience of monetary gains and losses. Neuron 30:619–639.

Breiter HC, Rosen BR (1999) Functional magnetic resonance imaging of brain reward circuitry in the human. Ann NY Acad Sci 877:523–547.

Brodie MS, Pesold C, Appel SB (1999) Ethanol directly excites dopaminergic ventral tegmental area reward neurons. Alcohol Clin Exp Res 23:1848–1852.

Bucholz KK, Cadoret R, Cloninger CR, Dinwiddie SH, Hesselbrock VM, Nurnberger JI Jr, Reich T, Schmidt I, Schuckit MA (1994) A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. J Stud Alcohol 55:149–158.

Dade LA, Zatorre RJ, Jones-Gotman M (2002) Olfactory learning: convergent findings from lesion and brain imaging studies in humans. Brain 125:86–101.

Doty RL (1995) *The Smell Identification Test: Administration Manual*. 3rd ed. Sensonics, Inc., Haddon Heights, NJ.

Duvernoy HM (1995) *The Human Brain Stem and Cerebellum*. Springer-Verlag, New York.

Copyright © Research Society on Alcoholism. Unauthorized reproduction of this article is prohibited.

George MS, Anton RF, Bloomer C, Teneback C, Drobes DJ, Lorberbaum JP, Nahas Z, Vincent DJ (2001) Activation of prefrontal cortex and anterior thalamus in alcoholic subjects on exposure to alcohol-specific cues. Arch Gen Psychiatry 58:345–352.

Grüsser SM, Heinz A, Flor H (2000) Standardized stimuli to assess drug craving and drug memory in addicts. J Neural Transm 107:715–720.

Guardia J, Catafau AM, Batlle F, Martin JC, Segura L, Gonzalvo B, Prat G, Carrio I, Casas M (2000) Striatal dopaminergic D-2 receptor density measured by [^{123}I]iodobenzamide SPECT in the prediction of treatment outcome of alcohol-dependent patients. Am J Psychiatry 157:127–129.

Hasin D, Paykin A (1999) Alcohol dependence and abuse diagnoses: concurrent validity in a nationally representative sample. Alcohol Clin Exp Res 23:144–150.

Hasin D, Van Rossem R, McCloud S, Endicott J (1997) Alcohol dependence and abuse diagnoses: validity in community sample heavy drinkers. Alcohol Clin Exp Res 21:213–219.

Hutchison KE, Swift R, Rohsenow DJ, Monti PM, Davidson D, Almeida A (2001) Olanzapine reduces urge to drink after drinking cues and a priming dose of alcohol. Psychopharmacologia 155:27–34.

Kareken DA, Mosnik D, Doty RL, Dzemidzic M, Hutchins G (2003) Functional anatomy of human odor sensation, discrimination, and identification in health and aging. Neuropsychology 17:482–495.

Kareken DA, Doty RL, Moberg PJ, Chen SH, Farlow MR, Mosnik DM, Hutchins G (2001) Olfactory-evoked regional cerebral blood flow in Alzheimer disease. Neuropsychology 15:18–29.

Katner SN, Kerr TM, Weiss F (1996) Ethanol anticipation enhances dopamine efflux in the nucleus accumbens of alcohol-preferring (P) but not Wistar rats. Behav Pharmacol 7:669–674.

Katner SN, Weiss F (1999) Ethanol-associated olfactory stimuli reinstate ethanol-seeking behavior after extinction and modify extracellular dopamine levels in the nucleus accumbens. Alcohol Clin Exp Res 23:1751–1760.

Katner SN, Weiss F (2001) Neurochemical characteristics associated with ethanol preference in selected alcohol-preferring and -nonpreferring rats: a quantitative microdialysis study. Alcohol Clin Exp Res 25:198–205.

Knutson B, Adams CM, Fong GW, Hommer D (2001) Anticipation of increasing monetary reward selectively recruits nucleus accumbens. J Neurosci 21:U1–U5.

Lorig TS, Elmes DG, Zald DH, Pardo JV (1999) A computer-controlled olfactometer for fMRI and electrophysiological studies of olfaction. Behav Res Methods Instrum Comput 31:370–375.

Lowe MJ, Sorenson JA (1997) Spatially filtering functional magnetic resonance imaging data. Magn Reson Med 37:723–729.

Mai JK, Assheuer J, Paxinos G (1997) *Atlas of the Human Brain*. San Diego, Academic Press.

O'Doherty JP, Deichmann R, Critchley HD, Dolan RJ (2002) Neural responses during anticipation of a primary taste reward. Neuron 33: 815–826.

Poellinger A, Thomas R, Lio P, Lee A, Makris N, Rosen BR, Kwong KK (2001) Activation and habituation in olfaction—an fMRI study. Neuroimage 13:547–560.

Price JL (1990) Olfactory system, in *The Human Nervous System* (Paxinos G ed), pp 979–998. Academic Press, San Diego.

Robinson TE, Berridge KC (1993) The neural basis of drug craving—an incentive-sensitization theory of addiction. Brain Res Rev 18:247–291.

Rohsenow DJ, Monti PM, Colby SM, Gulliver SB, Sirota AD, Niaura RS, Abrams DB (1997) Effects of alcohol cues on smoking urges and topography among alcoholic men. Alcohol Clin Exp Res 21:101–107.

Rothblat DS, Rubin E, Schneider JS (2001) Effects of chronic alcohol ingestion on the mesostriatal dopamine system in the rat. Neurosci Lett 300:63–66.

Saunders JB, Aasland OG, Babor TF, de la Fuente JR, Grant M (1993) Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption–II. Addiction 88:791–804.

Schneider F, Habel U, Wagner M, Franke P, Salloum JB, Shah NJ, et al. (2001) Subcortical correlates of craving in recently abstinent alcoholic patients. Am J Psychiatry 158:1075–1083.

Schultz W (2000) Multiple reward signals in the brain. Nat Rev Neurosci 1:199–207.

Sell LA, Morris J, Bearn J, Frackowiak RS, Friston KJ, Dolan RJ (1999) Activation of reward circuitry in human opiate addicts. Eur J Neurosci 11:1042–1048.

Smith AD, Weiss F (1999) Ethanol exposure differentially alters central monoamine neurotransmission in alcohol-preferring versus -nonpreferring rats. J Pharmacol Exp Ther 288:1223–1228.

Sobel N, Prabhakaran V, Zhao Z, Desmond JE, Glover GH, Sullivan EV, Gabrieli JD (2000) Time course of odorant-induced activation in the human primary olfactory cortex. J Neurophysiol 83:537–551.

Sobell MB, Sobell LC, Klajner F, Pavan D, Basian E (1986) The reliability of a timeline method for assessing normal drinker college students' recent drinking history: utility for alcohol research. Addict Behav 11: 149–161.

Stormark KM, Laberg JC, Bjerland T, Nordby H, Hugdahl K (1995) Autonomic cued reactivity in alcoholics: the effect of olfactory stimuli. Addict Behav 20:571–584.

Tapert SF, Cheung EH, Brown GG, Frank LR, Paulus MP, Schweinsburg AD, Meloy MJ, Brown SA (2003) Neural response to alcohol stimuli in adolescents with alcohol use disorder. Arch Gen Psychiatry 60:727–735.

Weinstein A, Lingford-Hughes A, Martinez-Rage J, Marshall J (1998) What makes alcohol-dependent individuals early in abstinence crave for alcohol: ezposure to the drink, images of drinking, or remembrance of drinks past? Alcohol Clin Exp Res 22:1376–1381.

Weiss F, Lorang MT, Bloom FE, Koob GF (1993) Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. J Pharmacol Exp Ther 267:250–258.

Weiss F, Porrino LJ (2002) Behavioral neurobiology of alcohol addiction: recent advances and challenges. J Neurosci 22:3332–3337.

West CHK, Clancy AN, Michael RP (1992) Enhanced responses of nucleus accumbens neurons in male rats to novel odors associated with sexually receptive females. Brain Res 585:49–55.

Wise RA (1998) Drug-activation of brain reward pathways. Drug Alcohol Depend 51:13–22.

Woods RP, Cherry SR, Mazziotta JC (1992) Rapid automated algorithm for aligning and reslicing PET images. J Comput Assist Tomogr 16:620–633.

Wrase J, Grusser SM, Klein S, Diener C, Hermann D, Flor H, Mann K, Braus DF, Heinz A (2002) Development of alcohol-associated cues and cue-induced brain activation in alcoholics. Eur Psychiatry 17:287–291.

Young AMJ, Ahier RG, Upton RL, Joseph MH, Gray JA (1998) Increased extracellular dopamine in the nucleus accumbens of the rat during associative learning of neutral stimuli. Neuroscience 83:1175–1183.

Zatorre RJ, Jones-Gotman M, Evans AC, Meyer E (1992) Functional localization and lateralization of human olfactory cortex. Nature 360: 339–340.

Copyright © Research Society on Alcoholism. Unauthorized reproduction of this article is prohibited.