

Effect of Naltrexone and Ondansetron on Alcohol Cue-Induced Activation of the Ventral Striatum in Alcohol-Dependent People

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Context: Medication for the treatment of alcoholism is currently not particularly robust. Neuroimaging techniques might predict which medications could be useful in the treatment of alcohol dependence.

Objective: To explore the effect of naltrexone, ondansetron hydrochloride, or the combination of these medications on cue-induced craving and ventral striatum activation.

Design: Functional brain imaging was conducted during alcohol cue presentation.

Setting: Participants were recruited from the general community following media advertisement. Experimental procedures were performed in the magnetic resonance imaging suite of a major training hospital and medical research institute.

Patients: Ninety non-treatment-seeking alcohol-dependent (by DSM-IV criteria) and 17 social drinking (< 14 drinks per week) paid volunteers recruited through advertisements at an academic center.

Interventions: A taste of alcohol and a series of alcohol-related pictures, neutral beverage pictures, and visual control images were provided to volunteers after 7 days of

double-blind randomly assigned daily dosing with 50 mg of naltrexone (n=23), 0.50 mg of ondansetron hydrochloride (n=23), the combination of the 2 medications (n=20), or matching placebos (n=24).

Main Outcome Measures: Difference in brain blood oxygen level-dependent magnetic resonance when viewing alcohol pictures vs neutral beverage pictures with a particular focus on ventral striatum activity comparison across medication groups. Self-ratings of alcohol craving.

Results: The combination treatment decreased craving for alcohol. Naltrexone with ($P=.02$) or without ($P=.049$) ondansetron decreased alcohol cue-induced activation of the ventral striatum. Ondansetron by itself was similar to naltrexone and the combination in the overall analysis but intermediate in a region-specific analysis.

Conclusions: Consistent with animal data that suggest that both naltrexone and ondansetron reduce alcohol-stimulated dopamine output in the ventral striatum, the current study found evidence that these medications, alone or in combination, could decrease alcohol cue-induced activation of the ventral striatum, consistent with their putative treatment efficacy.

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CONSIDERABLE DATA ARE available to support the use of the opiate antagonist naltrexone in the treatment of alcohol dependence.¹⁻³ Naltrexone is approved by the US Food and Drug Administration for the treatment of alcoholism and has been shown to reduce either the priming effect or the reward (stimulation) effect of alcohol.⁴⁻⁸ Also, in clinical treatment studies, naltrexone has been found to enhance abstinence,⁹ to reduce drinks per drinking day,^{1,2} to reduce craving,³ and to enhance resistance (reduce urge and impulse) to drink.^{1,10} Unfortunately, not all studies^{11,12} with naltrexone have been posi-

tive. In addition, a meta-analysis of 27 randomized controlled trials of naltrexone reported that, although short-term treatment with naltrexone decreased relapse, the number of patients needed to be treated to achieve a better outcome over placebo response was 7.¹³ This number needed to treat for a positive effect of naltrexone over placebo was recently confirmed in a large multisite trial, the Combined Pharmacotherapies and Behavioral Interventions for Alcohol Dependence (COMBINE) Study.¹⁴ This evidence suggests that not everyone responds to treatment with naltrexone. It is not clear whether naltrexone works by blocking cue-induced reinforcement as suggested by some animal^{15,16} and hu-

man^{17,18} studies or if it works primarily by blocking alcohol's pharmacologic reward properties.^{4,19-21}

The relative lack of robust data regarding the medication treatment of alcohol dependence has led to the idea of combining medications to improve treatment outcomes. The rationale is to use medications that target multiple neurotransmitter systems thought to be involved in alcoholism. One such study was the COMBINE Study in which naltrexone alone, acamprosate calcium alone, or the combination of the 2 medications was evaluated.¹⁴ Unfortunately, although no increased efficacy was found from the combination of the medications in this study, at least 1 smaller study²² suggested efficacy of combined naltrexone and acamprosate.

Although not approved by the US Food and Drug Administration, serotonin 3 antagonist drugs have produced evidence of their potential clinical utility in the treatment of alcoholism.^{23,24} Ondansetron hydrochloride is a serotonin 3 antagonist that has been found to have potential clinical utility in terms of animal studies²⁵ and human clinical laboratory paradigms.^{24,26} In clinical trials, Sellers et al²⁷ reported a greater reduction in drinks per drinking day in a subgroup of individuals treated with low-dose ondansetron hydrochloride (0.25 mg twice daily) compared with placebo or high-dose ondansetron hydrochloride (2.0 mg twice daily), and Johnson et al²⁸ found that ondansetron hydrochloride (4 µg/kg) reduced drinks per drinking day and increased abstinent days in individuals with early-onset alcoholism but not in those with late-onset alcoholism.

Secondary to the possible synergistic mechanisms on decreasing alcohol use, the combination of naltrexone and ondansetron has been studied in preclinical and clinical studies. Both rats and mice evaluated in a limited access paradigm had a greater reduction in alcohol intake when both medications were given together vs either medication alone.²⁹ In addition, an 8-week study in 20 individuals with early-onset alcoholism showed a significant difference in drinks per day between those who received naltrexone in combination with ondansetron and those who received placebo.³⁰

It has been thought that human alcohol cue-based laboratory paradigms might provide useful transitional data between animal laboratory support for potential alcohol treatment medications and clinical trials.^{4,19,21} However, the study of medication effects on alcohol cue response in the clinical laboratory is difficult secondary to the variability of subjective response (eg, craving) to cues and the variability in objective peripheral measures of autonomic arousal and response, such as heart rate changes and salivary output.³¹ In addition, these subjective and peripheral measures provide more distal and only correlative information as to what might be happening in the brain of the alcohol-addicted or alcohol-dependent individual. This has led us to seek a more proximal brain signal of alcohol cue-induced urges to drink and reward salience in which to explore medication effects as a potential predictor of treatment utility.

Several brain imaging technologies have been refined and applied to the study of brain activation during presentation of drug-related cues. Recent studies³²⁻³⁷ have indicated that similar findings may be emerging during

the presentation of alcohol cues. Although imaging studies have begun to shed light on the areas of the brain involved in alcohol craving, data regarding the impact of drug treatments on these structures are lacking.

An area of cue-stimulated activation noted by our group has been the ventral striatum.³⁵ The mesolimbic dopamine pathway that projects from the ventral tegmental area (VTA) to a structure within the ventral striatum, the nucleus accumbens (NAC), has been implicated as a major site for the reinforcing actions of many addictive drugs, including ethanol.³⁸⁻⁴² and naltrexone has been shown to block this effect.^{15,16,43} Therefore, the goals of the current study were to (1) replicate our previous findings that individuals with alcoholism have differential brain activation to alcohol cues compared with social drinkers, especially in the ventral striatum, and (2) explore, in a double-blind, placebo-controlled fashion, the effect of naltrexone, ondansetron, or the combination of the medications on cue-induced craving and ventral striatum activation. A priori hypotheses were that participants treated with naltrexone or ondansetron would have lower ventral striatum activation to alcohol cues compared with placebo-treated participants and that the combination of naltrexone and ondansetron would have a greater reduction in cue-induced craving and ventral striatum activation compared with participants treated with naltrexone or ondansetron alone.

METHODS

PARTICIPANTS

Non-treatment-seeking individuals (n = 125) who met the criteria for alcohol dependence participated in a larger protocol that included a limited-access, bar-laboratory paradigm for which general methods have been previously described.⁴ From this larger study, 100 participants agreed to take part in a brain imaging study. Of these 100 participants, 10 were excluded for the following reasons: head movement (2 participants), artifact (1), mechanical problems (1), incomplete craving ratings in the scanner (5), and a positive prescan breath alcohol level (1). Therefore, 90 non-treatment-seeking individuals had evaluable data for the analysis. Non-treatment-seeking individuals with alcoholism, after baseline evaluation, were assigned through urn randomization (using a double-dummy placebo-controlled design) to 1 of 4 experimental groups: naltrexone, ondansetron, naltrexone and ondansetron, or placebo. Participants received study drugs for 8 days (days 1-5 being a natural observation period). On day 7, after a minimum of 24 hours of abstinence, participants in the current study underwent functional magnetic resonance imaging (MRI) of the brain with cue stimulation. The bar-laboratory study took place on day 8. A smaller group of social drinker control subjects (n = 17) who were recruited and randomly assigned to the same medication groups and protocol were used as procedure controls as a comparison and contrast group for the brain imaging substudy.

Potential participants, recruited through newspaper and community advertisements based on drinking at least 20 drinks per week, were told that the study was investigating effects of medications that may have beneficial effects for people being treated for alcoholism. All participants met *DSM-IV* criteria for alcohol dependence, including loss-of-control drinking or an inability to cut down or quit, but they denied any active involvement in, or desire for, alcohol treatment. Exclusion criteria for

all participants were as follows: current *DSM-IV* criteria for drug dependence by verbal report and urine drug screens, other major *DSM-IV* Axis I disorders, psychoactive medication or substance use (except marijuana) in the past 30 days or a positive urine drug screen result, current suicidal or homicidal ideation, history of alcohol-related medical illness, liver enzyme levels 2.5 or more times above normal, or significant health problems. Participants who smoked more than 10 cigarettes a day were also excluded. All participants were screened for *DSM-IV* criteria with the use of the entire Structured Clinical Interview for all the *DSM-IV* Axis I Disorders (SCID).⁴⁴

PROCEDURES

When the participant arrived for the first session, the study was described in detail and informed consent was obtained by using a form and procedures approved by the investigational review board at our institution. Each participant was then evaluated with a number of standard interview, questionnaire, and medical diagnostic procedures similar to those in other studies reported by our group.^{4,19} Interview procedures included a demographic form, the alcohol and drug section of the SCID administered by a trained physician (K.V.), and a timeline follow-back interview to quantify drinking during the preceding 90 days.⁴⁵ The Obsessive-Compulsive Drinking Scale (OCDS),⁴⁶ the Self-administered Alcohol Screening Test,⁴⁷ and the Alcohol Dependence Scale⁴⁸ were administered. Finally, a urine specimen was collected to screen for abused drugs, and a blood sample was collected for liver function testing and general health screening. Additional assessments were conducted at a second session (conducted within 1 week of the first session), including psychiatric sections of the SCID. In addition, a physical examination was conducted by a physician assistant and reviewed by a physician (R.F.A.).

Participants who passed all screening and eligibility criteria were randomly assigned to receive 50 mg of naltrexone, 0.25 mg twice daily of ondansetron hydrochloride, 50 mg of naltrexone and 0.25 mg twice daily of ondansetron hydrochloride, or matching placebo. The medication regimen was for 8 days. Medication ingestion was witnessed on days 1, 6, and 7 by research staff. All medications, including inactive placebo, were blister packed and administered in standard gel caps with 25 mg of riboflavin added to assess for compliance via a laboratory-based urinary fluorescence assay. Urine samples were obtained and assessed for riboflavin at baseline and on day 7. Samples that showed greater than 1500 ng/mL of riboflavin were considered adherent to the drug regimen.⁴⁹

Participants were given no explicit instructions regarding use of alcohol or modification of their drinking behavior for days 1 through 5. However, they were required to abstain completely from drinking on days 6 and 7. On day 6, several assessments were completed. Participants were clinically evaluated for alcohol withdrawal using the Clinical Institute Withdrawal Assessment for Alcohol-Revised (CIWA-Ar).⁵⁰ A 6-day version of the timeline follow-back interview (in which participants reported their alcohol consumption since the outset of the medication period) was also completed. The symptom checklist and the OCDS were administered again. Participants were instructed to return the next day for the imaging session.

The cue-induced MRI procedures are similar to those used in previous work by our group.³⁵ Briefly, on the day of the imaging session, participants completed assessment questionnaires (timeline follow-back, OCDS, and CIWA-Ar), had their breath analyzed, and underwent a rapid urine drug screen. No participant had evidence of alcohol use or a positive urine drug screen result before the imaging session. They were then fitted

with 3-dimensional magnetic resonance-compatible goggles (Magnetic Resonance Technology, Northridge, California), and a 2-dimensional trackball was placed under their dominant hand. After positioning in the scanner, participants were checked to ensure that they could view the cues comfortably while wearing the goggles and were trained to rate their "urge to consume alcohol" by moving the trackball along a 100-mm analog scale anchored on one end as "not at all" and the other as "maximum possible." During initial scanner tuning and structural scanning (T1-weighted 3-dimensional volume and T1-weighted structural scan in the functional scan plane), participants were shown relaxation pictures. They were given a sip of preferred spirits in noncarbonated juice through a straw placed in their mouth and then shown the 12 minutes to 48 seconds of alternating stimuli with blood oxygen level-dependent (BOLD) image acquisition. Participants self-rated their craving in real-time (after each picture block) using the trackball during the picture viewing and brain image acquisition. After the imaging session, they were escorted out of the scanner room, rinsed their mouths with water, and underwent a breath analysis (the sip of alcohol does not produce measurable breath alcohol readings). They were then given instructions not to drink that evening, reminded of the next day's breath analysis and experiment, and sent home, either with a friend or family member or by taxi.

ALCOHOL CUES

These cue-induced MRI procedures are similar to those used in our previous work.³⁵ Briefly, alcoholic and nonalcoholic beverage picture cues were selected primarily from the Normative Appetitive Picture System ($n=38$) but were supplemented with 22 additional cues selected from advertisements to avoid repeating the same stimuli during the scanning sequence. Visual control pictures match the alcohol cues in color and hue but lack any object recognition. A sequence for stimulus presentation has been created consisting of six 120-second epochs. Each epoch contains four 24-second blocks (1 block each of alcoholic beverage, nonalcoholic neutral beverage, visual control pictures, and rest) and a 6-second alcohol craving rating after each block. Each 24-second block is made up of 5 individual pictures, each displayed for approximately 4.8 seconds. The alcohol blocks are specific to a beverage type (beer, wine, or liquor), with 2 blocks per type. The rest block consisted of a crosshair or plus sign shown for the duration of the 24-second block. To control for time and order effects across study participants, the order of the individual pictures, the blocks within the epoch, and the epochs are all randomly presented. After each 24-second block, participants were asked to rate their "urge to consume alcohol."

MRI ACQUISITION

Participants wore earplugs and head movement was restricted by cushions surrounding the head. The MRI was performed with a 1.5-T magnetic resonance scanner (Royal Philips Electronics, Amsterdam, the Netherlands) with actively shielded magnet and high-performance gradients (27 mT/m, 72 T/ms). An initial high-resolution, 142-section, 1-mm-thick, sagittal, T1-weighted scan was obtained for later volumetric and coregistration analysis and to ensure there was no significant anatomical brain disease. A structural scan was then obtained that consisted of 25 coplanar coronal sections (5 mm thick and 0-mm gap), covered the entire brain, and was positioned using a sagittal scout image. After another manual tuning for echoplanar imaging, the cue-induction paradigm was performed while also acquiring BOLD weighted coronal scans in the exact plane as

before using a gradient echo, echo-planar functional MRI (fMRI) sequence (tip angle, 90°; echo time, 27.0 milliseconds; repetition time, 3000 milliseconds; field of view, 27.0 cm; 25 sections 5 mm thick; and gap, 0.0 mm; with frequency selective fat suppression).

STATISTICAL ANALYSIS

Baseline Characteristics

Analyses of baseline drinking and demographics were performed with either analysis of variance (continuous variables) or χ^2 tests (categorical variables).

fMRI Data Analyses

The MRIs were transferred into ANALYZE format and then further processed on Sun workstations (Sun Microsystems, Palo Alto, California), using MATLAB 6.1 (MathWorks, Sherborn, Massachusetts) with Statistical Parametric Mapping software 2 (SPM2; The Wellcome Department of Cognitive Neurology, London, England; <http://www.fil.ion.bpmf.ac.uk>). Default settings were used unless indicated otherwise. All volumes were realigned to the first volume. After realignment (including the adjustment for sampling errors), for all participants movement across the entire scan was less than 1 mm in 3 axes and less than 1° in 3 orientations. Then, the images were stereotactically normalized into a standard space with a resolution of $3 \times 3 \times 3$ -mm voxels using the averaged functional echo-planar image (the Montreal Neurological Institute echo-planar imaging template in SPM2). Subsequently, the data were smoothed with an anisotropic $8 \times 8 \times 8$ -mm gaussian kernel and high-pass filtered (cutoff period, 240 seconds). This first level of statistical analysis used a boxcar function convolved with the modeled hemodynamic response function as the basic function for the general linear model. Contrast maps were obtained of the difference between alcohol minus beverage, alcohol minus visual control, alcohol minus rest, beverage minus visual control, and visual control minus rest for each patient individually, with the 6 head movement parameters included as covariates. The participant-specific contrasts were then entered into a second-level analysis to obtain a random-effects analysis of activation effects in the entire group. The combined group *t* maps were thresholded using an uncorrected $P \leq .001$ and a cluster statistical weight (spatial extent threshold) of 15 voxels.

The fMRI data were analyzed without knowledge of specific medication group assignment. The individual data were divided into 5 groups (corresponding to 4 medication and 1 social drinker group without specific identification of treatments applied). To identify activity in the ventral striatum among all participants, conjunction analysis⁵¹ was performed with multiple regression (no constant term) in basic models. The voxel location of the highest *t* value (uncorrected $P < .001$) was used to create a mask for time course extraction. A small volume of 6-mm-radius spherical regions of interest was used to create a mask within the ventral striatum that was centered at the location (Montreal Neurological Institute coordinates) of the right nucleus accumbens (9, 6, -8). With the mask, averaged time courses of multivoxels were generated from each individual datum.

Specific Effects of Medication on Alcohol Cue-Induced Ventral Striatum Activation

Analysis of the ventral striatum data was performed as a 3-level hierarchical linear regression (HLM 6.04) with time (level 1) nested within condition (level 2) nested within participant (level

3). Level 2 predictors, the dummy-coded variables that represent the contrasts of beverage relative to each of the other conditions (rest, visual control, and alcohol), were analyzed as random variates. Level 3 predictors were those distinguishing between participants (ie, drug group) and any constant participant-level covariate (eg, age). This a priori defined analysis focused on the differential effects of each of the between-participant variables (different medication conditions) on the difference in ventral striatum activation generated during neutral beverage vs alcohol visual stimuli (the dependent variable of interest). The medication conditions were represented with indicator-coded dummy variables using the placebo group as a reference. The analysis further allowed overall tests of whether the beverage-to-alcohol ventral striatum activation difference varied across the sequential alcohol picture blocks of the experimental protocol (block \times alcohol interaction).

Although preliminary analyses revealed a modest increase in ventral striatum activation (BOLD response) across blocks (time) ($t_{98} = 2.2$; $P = .03$), this was the same for all picture stimuli contrasts (for all block \times condition interactions $P > .65$) and neither the block (time) effect nor interactions varied with medication group ($P > .25$ for all). Furthermore, in no case was the pattern of significant contrast effects altered by the inclusion of any block (time)-related variables. In summary, although individual variation was found in low-frequency drift, the group relationships stayed constant across the experimental session and are reported herein.

Craving Analysis

Analysis of craving scores was performed in a hierarchical linear regression similar to that used for ventral striatum activation but was only a 2-level model, stimulus condition nested within participant. The primary analysis was directed at estimating the difference in craving during the beverage vs alcohol conditions. Estimates of the alcohol minus beverage contrast for ventral striatum activation were calculated from the bayesian residuals of the overall hierarchical model and were compared (using standard regression techniques) with the overall craving experienced by the participants while in the scanner.

RESULTS

DEMOGRAPHICS AND SUBJECTIVE RATINGS

As indicated in **Table 1**, no significant baseline differences were found in demographics or alcohol use parameters among the medication groups. However, as designed, a significant difference was found among the medication groups and social drinker groups in drinking parameters ($P < .001$). No alcohol withdrawal symptoms were evident in any group because the CIWA-Ar scores were zero. In addition, the results of urine drug screens performed before the scanning session were negative. Medication compliance in all groups was greater than 90% by urinary riboflavin and pill counts. Two items in the symptom checklist discriminated among the treatment groups. "Nausea-vomiting" and "dizziness" were significantly higher in participants receiving naltrexone than in those receiving ondansetron alone or placebo (overall group effect: $\chi^2_3 = 16.9$, $P = .001$; and $\chi^2_3 = 12.1$, $P = .007$; for nausea and dizziness, respectively). Both were largely absent in the group receiving ondansetron alone (2 of 23 and 0 of 23 for the 2 adverse effects, respec-

Table 1. Demographics and Drinking History^a

Demographics	Naltrexone Group (n=23)	Ondansetron Hydrochloride Group (n=23)	Combination Group (n=20)	Placebo Group (n=24)	Control Group (n=17)
Age, y	27.22 (8.84)	25.13 (6.88)	26.15 (8.82)	24.75 (5.74)	25.18 (4.00)
Male, No. (%)	17 (74)	15 (65)	14 (70)	18 (75)	14 (82)
White, No. (%)	20 (87)	22 (96)	17 (85)	21 (88)	16 (94)
Education, y	14.65 (1.87)	14.43 (1.47)	15.40 (1.14)	13.25 (2.07)	16.24 (1.30)
Drinks per drinking day	11.33 (6.39)	9.64 (5.43)	8.31 (3.68)	9.19 (3.90)	3.18 (1.82)
Drinks in the past month	205.92 (88.78)	227.48 (76.95)	202.26 (88.69)	196.74 (84.67)	24.96 (18.35)
OCDS total score	17.52 (6.70)	18.64 (6.43)	16.65 (5.74)	19.42 (7.42)	2.94 (1.89)
SAAST total score	3.22 (1.24)	3.52 (1.24)	3.50 (1.47)	3.48 (1.72)	2.13 (0.04)
ADS total score	14.09 (6.39)	13.70 (6.95)	14.05 (5.36)	16.10 (6.95)	1.57 (2.21)

Abbreviations: ADS, Alcohol Dependence Scale; OCDS, Obsessive-Compulsive Drinking Scale; SAAST, Self-administered Alcohol Screening Test.

^aData are presented as mean (SD) unless otherwise indicated. Differences between treatment conditions were nonsignificant. Drinking history and drinking measure (OCDS, SAAST, and ADS) scores in the control group were significantly different from those in the treatment groups ($P < .01$).

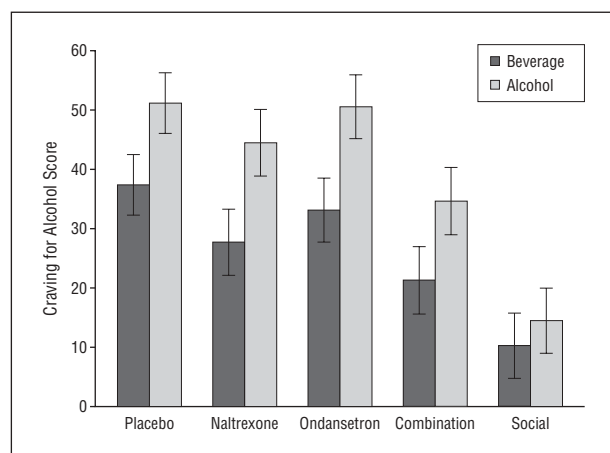


Figure 1. Subjective craving for alcohol and beverage cues were rated on a scale of 0 to 100 within the scanner. Study participants treated with the combination of naltrexone and ondansetron hydrochloride had significantly less craving for alcohol compared with placebo-treated participants ($P = .04$). In addition, social drinking controls had less craving for alcohol compared with placebo-treated participants ($P = .001$). Bars indicate mean values; error bars, standard error of the mean.

tively), and the incidence did not differ from the placebo (for nausea, $P = .19$, and for dizziness, $P = .27$). Neither symptom significantly predicted ventral striatum activation when entered as a covariate ($t_{90} = 1.15$, $P = .25$; and $t_{90} = 1.4$, $P = .17$) or significantly changed the group relationship in the analysis.

CRAVING

As seen in **Figure 1**, significant differences were found among the groups with regard to the craving ratings during visual presentation within the scanner. As expected, social drinkers had reduced craving compared with the placebo-treated non-treatment-seeking individuals with alcoholism ($P = .001$). Non-treatment-seeking individuals with alcoholism treated with the combination of naltrexone and ondansetron had significantly reduced craving while viewing the alcohol pictures within the scanner compared with participants treated with placebo ($P = .04$). No significant differences

were found in craving scores between the placebo and naltrexone groups ($P = .20$) or between the placebo and ondansetron groups ($P = .56$).

COMPARISON OF ALCOHOL CUES WITH BEVERAGE CUES

The brain areas that were significantly activated within each group during the comparison of alcohol cues and beverage cues by SPM2 analysis are summarized in **Table 2** and depicted in **Figure 2**. Consistent with our previous study,³⁵ the placebo-treated non-treatment-seeking individuals with alcoholism had activation in prefrontal and limbic regions, areas not activated in social drinkers. Confirming our a priori hypothesis, individuals with alcoholism who were treated with naltrexone, either alone or in combination with ondansetron, did not experience the ventral striatum activation seen in the placebo-treated individuals with alcoholism. In addition, ventral striatum activation was not detected in the ondansetron-treated individuals with alcoholism in this analysis. The brain areas significantly activated in the medication groups vs those activated in the placebo group during the comparison of alcohol cues and beverage cues are listed in **Table 3**.

COMPARISONS OF OTHER CUES

The brain areas significantly activated during the comparison of alcohol cues with visual control cues are listed in Table 2. Similarity to areas activated in the alcohol cue or beverage cue comparison was found in non-treatment-seeking individuals with alcoholism, which was different from social drinkers, who had minimal salient alcohol cue activations.

VENTRAL STRIATUM ACTIVATION

Activation in the ventral striatum as defined by the region of interest HLM analysis in the various medication and social drinking controls is shown in **Figure 3**. A significant difference was found between the placebo-treated individuals with alcoholism and social drinkers ($t_{96} = 3.4$; $P = .001$). Also, within the group of individuals with

Table 2. Brain Areas Activated by Comparison

Group and Side	x	y	z	t Score	Region
Alcohol-Beverage Comparison					
Naltrexone					
L	-8	43	53	4.44	Superior medial frontal
R	3	-92	16	5.27	Cuneus
R	4	-95	12	7.83	Calcarine
Ondansetron ^a					
R	29	-88	24	4.29	Occipital
R	8	-68	3	4.07	Lingual
Combination					
R	5	62	31	6.05	Superior medial frontal
L	-1	26	31	4.37	Anterior cingulate
L	-45	-77	24	5.08	Middle occipital
R	34	-81	27	4.22	Middle occipital
R	4	-91	21	4.51	Cuneus
L	-1	-61	17	8.01	Calcarine
L	-39	27	-14	6.54	Inferior orbital frontal
R	3	-79	-16	4.43	Cerebellum
Placebo					
L	-18	41	40	5.49	Superior frontal
L	-2	-37	35	4.56	Middle cingulate
R	1	90	20	5.29	Cuneus
L	-3	-57	16	4.93	Calcarine
R	5	63	18	5.53	Superior medial frontal
R	17	-18	1	4.13	Thalamus
R	1	59	-3	4.03	Midfrontal
R	8	7	-8	5.01	Ventral striatum
L	-21	-23	-14	4.25	Parahippocampus
R	14	69	-15	4.93	Cerebellum
R	29	-42	-18	4.77	Fusiform
Social drinking					
L	-7	-99	9	3.99	Calcarine
Alcohol-Visual Control Comparison					
Naltrexone					
L	-9	63	30	4.26	Superior medial frontal
R	4	-94	7	4.96	Calcarine
L	-24	-93	-15	7.52	Lingual
R	25	-78	-18	5.85	Cerebellum
Ondansetron					
L	-13	39	51	4.87	Superior frontal
L	-25	-76	43	4.23	Occipital
R	1	3	35	4.17	Anterior cingulate
L	-7	60	32	4.15	Superior medial frontal
R	32	-90	20	5.52	Occipital
L	-1	-69	12	6.44	Calcarine
R	23	-25	-11	4.91	Hippocampus
L	-23	-32	-12	4.87	Parahippocampus
Combination					
L	-10	50	46	4.64	Superior medial frontal
R	17	48	45	4.39	Superior frontal
R	11	-94	20	4.72	Occipital
R	2	-91	7	6.42	Calcarine
L	-23	-28	-10	4.51	Hippocampus

(continued)

alcoholism, those who received placebo had significantly more alcohol cue-induced ventral striatum activation than did those treated with naltrexone ($t_{96}=2.0$; $P=.049$) or the combination of naltrexone and ondansetron ($P=.02$). However, those treated with ondansetron were intermediate between the placebo and other drug groups, being nonsignificantly ($t_{96}=1.05$; $P=.30$) lower than the placebo group but not as low as the groups taking naltrexone with or with-

Table 2. Brain Areas Activated by Comparison (cont)

Group and Side	x	y	z	t Score	Region
Alcohol-Visual Control Comparison (cont)					
Placebo					
L	-2	36	51	5.51	Superior medial frontal
L	-20	-81	45	4.76	Cuneus
L	-17	47	38	4.67	Superior frontal
R	1	-91	17	4.94	Cuneus
L	-8	53	9	4.31	Anterior cingulate
L	-15	4	3	5.07	Pallidum
R	1	-82	-2	7.65	Calcarine
R	9	6	-8	4.78	Ventral striatum
L	-26	-29	-12	4.49	Parahippocampus
R	25	-30	-14	4.67	Parahippocampus
L	-35	30	-18	4.85	Inferior orbital frontal
R	39	-69	-22	5.25	Cerebellum
L	-31	-49	-22	5.46	Cerebellum
Social drinking					
L	-19	-100	-10	4.16	Lingual
Beverage-Visual Control Comparison					
Naltrexone					
No activation					
Ondansetron					
R	17	-18	70	5.65	Precentral gyrus
Combination					
R	28	-96	-4	4.56	Occipital
L	-15	-101	-8	5.83	Lingual
Placebo					
L	-14	-100	-10	5.41	Lingual
R	27	-82	-13	4.4	Lingual
R	30	-82	-20	4.82	Cerebellum
Social drinking					
L	24	-69	-19	6.09	Cerebellum
R	21	-30	-12	4.02	Parahippocampus
L	-21	-95	-10	5.56	Lingual
R	18	-93	-6	7.68	Occipital

^aOndansetron given as ondansetron hydrochloride.

out ondansetron ($t_{96}=0.56$, $P=.58$ vs naltrexone; and $t_{96}=0.91$, $P=.37$ vs the combination). Thus, although this between-medication group HLM analysis of ventral striatum activation showed medication effects in the same direction as in the within-medication group SPM2 analysis, a more powerful effect for naltrexone than for ondansetron emerged in the HLM analysis.

RELATIONSHIP OF CRAVING WITH VENTRAL STRIATUM ACTIVATION

A strong curvilinear relationship was found across groups between the mean craving for alcohol during the scanning session and the mean of the alcohol minus beverage comparison (**Figure 4**). Linear regression of craving scores against the log of the alcohol or beverage ventral striatum activation was highly significant ($B=0.04$; $SE=0.005$; $P=.02$), with mean activation explaining more than 95% of the variance in the craving group means.

COMMENT

Although others have used fMRI neuroimaging technology to evaluate medication effects in individuals with al-

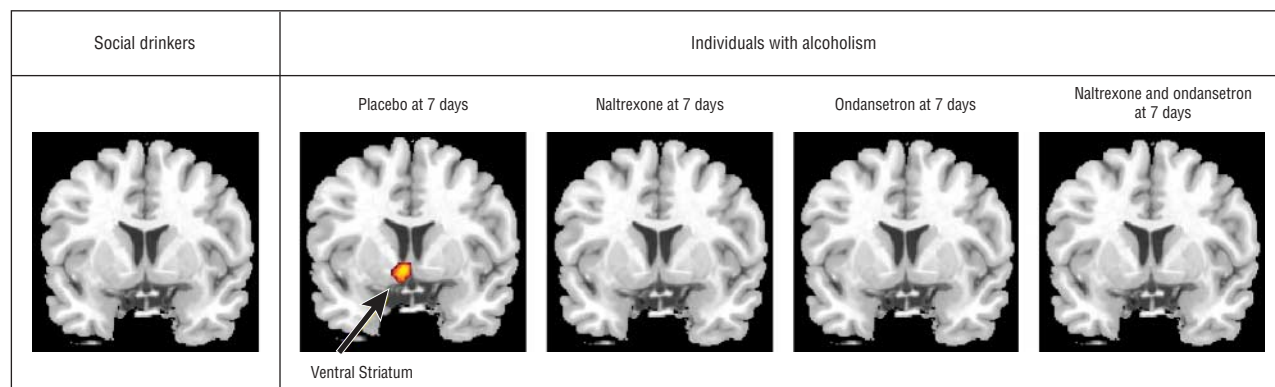


Figure 2. Brain regions with significantly increased activation in one task (alcohol) compared with another (beverage) are depicted in color on coronal structural magnetic resonance images ($P \leq .001$). Ondansetron given as ondansetron hydrochloride.

Table 3. Brain Areas Activated: Between-Medication Group Comparisons by Cue Condition^a

Group and Side	x	y	z	t Score	Region
Between-Medication Group Comparison of Alcohol-Beverage Conditions					
Placebo vs naltrexone					
R	6	6	-8	1.67	Ventral striatum
R	46	51	0	1.91	Orbital frontal
R	59	-38	35	2.13	Supramarginal
L	-22	59	-3	2.53	Supraorbital frontal
R	6	60	15	1.69	Medial frontal cortex
Placebo vs ondansetron ^b					
R	1	-39	-9	2.04	Vermis
R	3	-95	9	2.30	Calcarine
R	25	46	39	1.94	Superior frontal
L	-1	35	47	2.02	Middle cingulate
Placebo vs combination					
R	25	46	36	3.73	Middle frontal
R	4	6	-8	2.17	Ventral striatum
R	4	15	8	2.83	Caudate
Between-Medication Group Comparison of Alcohol-Visual Control Conditions					
Placebo vs naltrexone					
R	43	16	44	2.79	Middle prefrontal
L	-30	27	11	2.17	Insula
L	-11	0	18	3.01	Parahippocampus
R	33	17	4	1.74	Inferior frontal
R	7	6	-7	2.69	Ventral striatum
Placebo vs ondansetron					
R	42	19	42	2.59	Middle prefrontal
L	-24	59	34	2.74	Occipital
L	-37	14	3	1.75	Insula
L	-33	53	-1	2.69	Middle orbital prefrontal
L	-17	57	-24	2.62	Cerebellum
Placebo vs combination					
R	24	29	34	2.96	Superior frontal
R	13	57	12	2.72	Superior medial frontal
R	35	21	0	2.72	Insula
R	1	-45	69	3.18	Precuneus

^a $P = .05$ for 15 voxels.

^b Ondansetron given as ondansetron hydrochloride.

coholism,⁵² to our knowledge, this is the first study that used fMRI to evaluate alcohol cue-induced changes in regional brain activity, along with subjective reports of craving, during double-blind medication treatment. The results indicate that participants treated with the com-

bination of naltrexone and ondansetron had significantly less craving while viewing alcohol cues within the scanner compared with placebo-treated participants. Unexpectedly, no difference was found in craving while viewing the alcohol cues within the scanner between the placebo and naltrexone alone groups or between the placebo and ondansetron alone groups.

Our a priori hypothesis was that alcohol cue induction would result in activation of the ventral striatum in the placebo group in contrast to social drinkers. Consistent with our previous report,³⁵ a significant difference was found in ventral striatum activation between placebo treatment and social drinking controls ($P = .001$). Furthermore, we hypothesized that there would be reduction in this ventral striatum activation by both naltrexone and ondansetron and a greater reduction in ventral striatum activation in the combination treatment group than in either medication group alone. Consistent with that hypothesis, in our region of interest analysis, the most significant decrease in alcohol cue-induced ventral striatum activation compared with placebo treatment was observed in the naltrexone and ondansetron group ($P = .02$). Although naltrexone alone suppressed activation more than did placebo treatment, the difference was less robust ($P = .049$) and ondansetron alone caused a nonsignificantly lower activation than did placebo.

The ventral striatum contains the NAC, which is considered one of the primary neural substrates mediating addiction.^{53,54} It has been implicated in the rewarding properties of reinforcing behaviors and substances of abuse and has extensive cortical and subcortical connections. Systemic and oral ethanol administration increases the dopamine concentration in the NAC.⁵⁵⁻⁶⁰ Dopaminergic projections from the VTA to the NAC fire in response to presentation of reward cues and reward anticipation,^{41,61-63} and human positron emission tomography studies have implicated striatal dopamine systems in alcohol effects.⁶⁴⁻⁶⁶

Alcohol-associated cues (light or environmental) have signaled an increase in NAC dopamine output before actual alcohol consumption in animals.^{16,67} Because participants in our cue paradigm do not attain a measurable blood alcohol level, the alcohol taste and visual cue activation of the ventral striatum is consis-

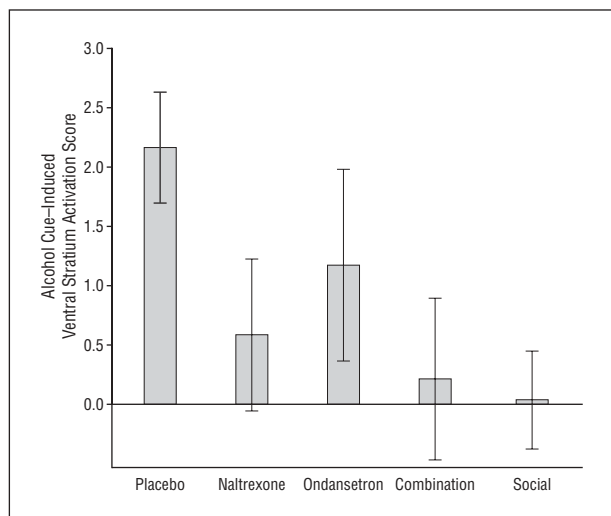


Figure 3. Ventral striatum activation (contrast of alcohol cue activation minus beverage cue activation) was significantly decreased in the combination naltrexone and ondansetron hydrochloride group ($P=.02$), the naltrexone alone group ($P=.049$), and the social drinking controls ($P=.001$) compared with the placebo-treated participants. Bars indicate mean values; error bars, standard error of the mean.

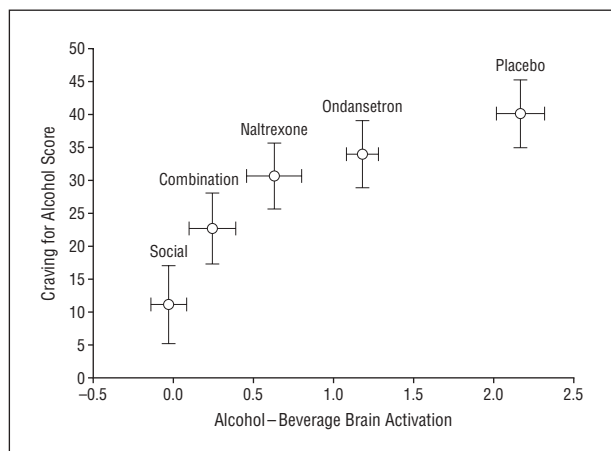


Figure 4. There is a strong curvilinear relationship across groups between the mean craving for alcohol during the scanning session (rated on a scale of 0 to 100) and the mean of the alcohol minus beverage comparison ($B=0.04$; $SE=0.005$; $P=.02$) in the ventral striatum. Horizontal and vertical error bars indicate standard error of the mean. Ondansetron given as ondansetron hydrochloride.

tent with alcohol cue stimulation of NAC dopamine release in animals. Other fMRI studies in humans found that anticipation of increasing monetary rewards in healthy volunteers yielded increasing NAC activation⁶⁸ and that memory of rewarding stimuli was preceded by differential activation of the VTA and NAC,⁶⁹ both consistent with our finding of increased salience of alcohol cue activation of these areas in alcohol-dependent individuals. Of interest, naltrexone has been found to block the NAC-activated dopamine release in anticipation of drinking and actual alcohol consumption.^{15,16,43} Taken together, these data suggest that naltrexone might be capable of disrupting “alcohol-induced reward memory” in individuals with alcoholism, leading to reduced cue responsiveness, craving, and relapse. The

addition of ondansetron to alcohol might enhance this effect.

It is thought that the serotonin 3 receptor interacts with dopamine cells in the VTA-NAC reward pathway. Serotonin 3 agonists can stimulate dopamine release in the NAC and also augment the ethanol-induced release of dopamine.^{58,70} This effect is blocked by serotonin 3 antagonists.^{58,70,71} The effects of the serotonin 3 antagonists are similar to those of naltrexone in these models,⁴³ suggesting that the 2 drugs may have synergistic actions.⁷²

Of note, the findings in the placebo group are consistent with our previous work^{33,35} and other published cue-induced imaging studies^{32,34,36,37} that involve substances of abuse. Regions activated include both limbic and cortical areas. These areas include various portions of the cingulate gyrus,⁷³⁻⁸⁰ the orbital cortex,^{77,79,81} and the ventral striatum.^{33,73,78}

Although in our hands the ventral striatum seems to be most affected by alcohol cue-induced activation, these others areas might play a significant role in reinforced memories, the subjective desire to drink, and perhaps attempts to resist urges and thoughts of drinking. These issues require further exploration.

In summary, the current study provides further evidence of the utility of neuroimaging techniques not only to further our understanding of the neurobiological basis of alcoholism but also to serve as a tool to provide crucial information regarding therapeutic manipulations of these underlying substrates of addiction. As such, neuroimaging can provide a bridge between preclinical and clinical work. Consistent with animal data that suggest that both naltrexone and ondansetron reduce alcohol-stimulated dopamine output in the ventral striatum, the present study found evidence that these medications could decrease cue-induced activation of the ventral striatum. The relationships among this deactivation, craving, alcohol consumption, and relapse drinking during treatment all require further exploration. In addition, individual differences in medication effects on alcohol cue brain deactivation, such as genetic makeup, age at onset of alcohol drinking and dependence, severity of dependence, sex, and racial/ethnic differences, are all worthy of future study.

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