Original Investigation

Disrupted Ventromedial Prefrontal Function, Alcohol Craving, and Subsequent Relapse Risk

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IMPORTANCE Alcohol dependence is a chronic relapsing illness; stress, alcohol-related cues, and neutral-relaxing states significantly influence craving and relapse risk. However, neural mechanisms underlying the association between these states and alcohol craving and relapse risk remain unclear.

OBJECTIVES To identify neural correlates associated with alcohol craving and relapse outcomes in 45 treatment-engaged, 4- to 8-week abstinent alcohol-dependent (AD) patients, and to compare brain responses of 30 demographically matched AD patients and 30 healthy control subjects during stress, alcohol, and neutral-relaxing cues.

DESIGN Functional magnetic resonance imaging study while participants were engaging in brief individualized script-driven imagery trials of stress, alcohol cues, and neutral-relaxing scenarios, and a prospective clinical outcome design to assess alcohol relapse 90 days postdischarge from inpatient treatment in the AD group.

SETTINGS Inpatient treatment setting in a community mental health center and hospital-based research unit.

PATIENTS Forty-five recovering AD patients in inpatient treatment for examining relapse, and 30 healthy control subjects demographically matched to 30 AD patients (subgroup of the relapse sample) for group comparisons.

INTERVENTION Twelve-step recovery–based addiction treatment for the patient group.

MAIN OUTCOMES AND MEASURES Brain response, alcohol craving, and relapse outcome measures (time to relapse and relapse severity).

RESULTS Increased ventromedial prefrontal cortex (vmPFC) and anterior cingulate cortex (ACC) activation during neutral-relaxing trials was correlated with high alcohol cue–induced and stress-induced craving in early recovering AD patients ($x = 6$, $y = 43$, $z = -6$; $P < .01$, whole-brain corrected). This vmPFC/ACC hyperactivity significantly predicted subsequent alcohol relapse, with a hazards ratio greater than 8 for increased relapse risk. Additionally, vmPFC/ACC hyperactivation during neutral trials and reduced activity during stress trials were each predictive of greater days of alcohol used after relapse ($P < .01$, whole-brain corrected). In contrast, matched control subjects showed the reverse pattern of vmPFC/ACC responses to stress, alcohol cues, and relaxed trials ($F = 6.42$; $P < .01$, whole-brain corrected).

CONCLUSIONS AND RELEVANCE Findings indicate that disrupted vmPFC/ACC function plays a role in jeopardizing recovery from alcoholism and may serve as a neural marker to identify those at risk for alcohol relapse.

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Alcohol dependence is a chronic, relapsing illness, which contributes to significant global disease burden.[1] The risk for relapse further perpetuates this disease burden and is increased in stress-related and alcohol-related contexts that promote anxiety and alcohol craving.[2-6] Neuroendocrine studies have demonstrated that upregulated hypothalamic-pituitary-adrenal axis response during a neutral-relaxing condition and blunted cortisol responses to stress significantly contribute to chronic alcoholism and relapse.[6-8] Evidence from electrophysiological studies also indicates disrupted electroencephalographic responses at rest during early recovery from alcoholism. For example, excessive alcohol use leads to neuroadaptations akin to a kindling-like process,[9] resulting in a hyperexcitable neuronal state, particularly in frontal regions.[10,11] Furthermore, alcohol-related neuroadaptations increase stress sensitivity[2,12] and stress-related anxiety response.[13] These studies suggest the importance of examining neural mechanisms of stress and nonemotional, neutral-relaxing states in assessing their contribution to alcohol relapse risk.

Although recent advances in neuroimaging techniques have provided insights into neuronal abnormalities in brain structure and function associated with chronic alcoholism, research on functional mechanisms associated with alcohol relapse has been rare and primarily focused on alcohol cue-related neural processes in the mesocortical-limbic pathways,[14] with little attention to neural mechanisms involved in stress and neutral-relaxing states and their associations with alcohol craving and relapse. Because chronic alcohol-related neuroadaptations target prefrontal networks that include the corticostriatal motivation pathways,[15] such neuroadaptations could promote increased craving and relapse risk.[2,6,12,16] Alcohol-related dysfunction in frontal networks might particularly affect higher order executive function including response inhibition and decision-making functions.[17-19] Furthermore, chronic alcohol-related hyperactivity of the mesolimbic dopamine-related impulsive pathways may further compromise the prefrontal self-control regions involved in the regulation of cravings and the will to resist relapse.[17,19,20] However, the specific role of the prefrontal regions and their interconnected networks in alcohol craving and relapse risk in the context of stress and neutral-relaxing states is not known.

Integrating the previously mentioned theoretical and empirical perspectives, the current study aimed to identify neural correlates of alcohol craving and future relapse risk in the context of stress, alcohol cue, and neutral-relaxing situations in recovering alcohol-dependent (AD) patients using a combined functional magnetic resonance imaging (fMRI) and prospective clinical design study. We examined all 3 conditions known to influence alcoholism in previous studies to investigate patterns of differential neural responses during these conditions. The neutral-relaxing condition was included as an active comparison state because it does not increase alcohol craving,[6] but it provides a context to assess changes in a resting-relaxed state while controlling for the nonspecific effects of the experimental manipulation. In previous work, we identified disrupted neuroendocrine responses in the relaxed states in AD patients with a strong association with alcohol relapse.[6] A second aim was to identify whether the same neural responses that are predictive of alcohol relapse show differences in brain responses when comparing recovering AD patients and demographically matched healthy control (HC) subjects.

For the relapse aim, 45 inpatient treatment-engaged, 4- to 8-week-abstinent, recovering AD individuals participated in an fMRI session. Participants were discharged from inpatient treatment following the fMRI session and prospectively followed up with repeated face-to-face assessments at days 14, 30, and 90 to assess relapse risk (**Figure 1**A). Relapse risk was assessed with the frequently used clinical outcome measures of time to first drink (relapse), time to heavy drinking relapse (5 or more drinks/occasion in men; 4 or more drinks/occasion in women), and alcohol relapse severity as measured by frequency of drinking days after first relapse. For group comparisons, we compared brain responses of age-matched, sex-matched, and intelligence-matched, right-handed, abstinent AD patients (a subgroup of the relapse sample) vs healthy, socially drinking individuals (30 in each group; eTable 1 in Supplement).

A block design approach that used a well-validated, individually calibrated, script-driven guided-imagery procedure (see Sinha article[21] for review) was implemented to experimentally induce challenging stress and alcohol cue states and an active neutral-relaxing control state via exposure to 6 brief trials of 2 stress, 2 alcohol cue, and 2 neutral-relaxing scenarios (different scripts presented in randomized order). The same fMRI procedures were used with both the AD patients and HC subjects in the study.

Methods

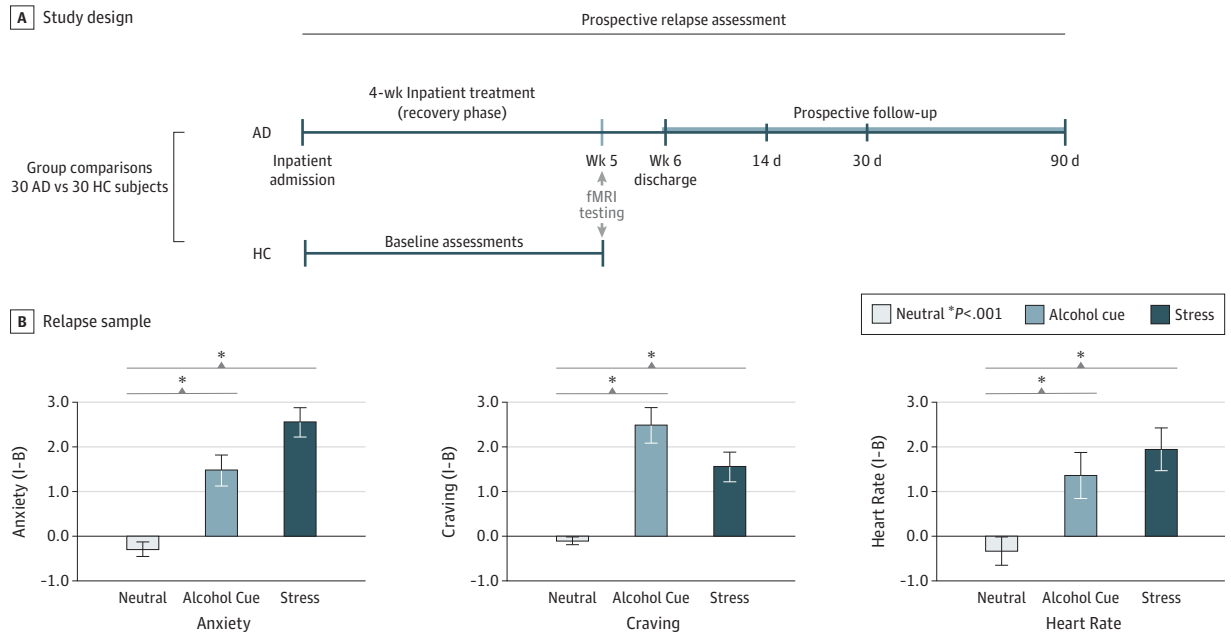
Participants

Forty-five recovering AD patients (10 female; aged 18-50 years) who had abstained from alcohol for 4 to 8 weeks (mean [SD], 34 (7.6) days; range, 29-59 days) residing in an inpatient treatment research facility and actively engaged in substance abuse treatment were recruited. All AD participants had a current diagnosis of alcohol dependence as determined by a Structured Clinical Interview for *DSM-IV*, with self-reported excessive alcohol use prior to inpatient admission and verified by urine toxicology testing on admission. For group comparisons, 30 HC subjects were demographically matched to 30, 4- to 8-week-abstinent AD patients (selected from the 45 previously described). The two groups were matched on age, intelligence as measured by the Shipley Institute of Living Scale,[22] handedness (all right handed based on self-report), sex ratio, and lifetime prevalence of psychiatric disorders except for alcoholism (eTable 1 and eAppendix in Supplement).

Individualized Imagery Method and Script Development

Prior to the fMRI session, individually tailored, 2-minute imagery scripts were developed based on the description of participants via standardized, structured interviews using Scene Construction Questionnaires (adopted from studies by Sinha[21] and Miller et al[23]) as described and validated in previous

Figure 1. Study Design



A, For relapse sample, all 45 alcohol-dependent (AD) patients resided in an inpatient treatment research facility for 6 weeks with functional magnetic resonance image (fMRI) testing in week 5, and patients were assessed with follow-up interviews at 14, 30, and 90 days following inpatient treatment discharge. For group comparisons, neural responses of 30 healthy control (HC) subjects were compared with 30 demographically matched AD patients from the relapse sample. B, Relapse sample including 45 AD patients. Mean behavioral ratings (and standard errors) in response to stress (S), alcohol cue (AC), and neutral-relaxing (N) imagery relative to baseline (imagery minus baseline [I − B] rating) for anxiety (S > N: $t = 8.0$, $P < .001$; AC > N: $t = 4.9$, $P < .001$; and S > AC: $t = 3.1$, $P < .01$) and alcohol craving (S > N: $t = 4.7$, $P < .001$; AC > N: $t = 7.1$, $P < .001$; and AC > S: $t = 2.4$, $P < .05$) before and after each trial using 10-point Likert scale verbal ratings. Averaged heart rate increases from baseline during stress ($t = 5.7$, $P < .001$) and alcohol cue ($t = 4.1$, $P < .001$) relative to neutral-relaxing trials. The dependent measures (I − B) were averaged across 2 trials of the same type (alcohol cue, stress, and neutral).

studies.[21] Two individualized scripts, each for stress, alcohol cue, and neutrally relaxing states were developed. For stress scripts, participants described their recent experience of most distressing situations, and only those situations rated as 8 or above (on a 10-point Likert scale with 10 = the most stressful) were selected for script development. Alcohol cue scripts were developed from personal experiences of alcohol-related situations that led to subsequent alcohol consumption (eg, seeing others drinking alcohol or meeting friends at a bar). Neutral scripts were developed based on commonly experienced, individual neutral-relaxing situations (eg, laying on the beach and listening to the waves or sitting in a park and reading on a Sunday afternoon). The script style, content format, and length were standardized across conditions and subjects, while preserving individual stimulus and response content specific to the individual experience as previously described.[21] Each 2-minute script was audiotaped and presented in random order during the scanning session (see eAppendix in Supplement for method description and sample scripts; eTable 2 in Supplement).

Functional Magnetic Resonance Imaging Acquisition and Procedure

Magnetic resonance imaging data were collected using a 3-T Siemens Trio MRI system equipped with a standard quadrature head coil, using T2*-sensitive gradient-recalled single-shot echo planar pulse sequence (see eAppendix in Supplement for fMRI parameters).

Six fMRI trials (2 per condition) were acquired using a block design. The order of 3 script conditions were randomized and counterbalanced across subjects. Each script was presented only once for a subject, and scripts in the same condition were not presented consecutively. Each trial lasted 5 minutes, including a 1.5-minute quiet baseline period followed by 2.5-minute imagery (2 minutes of read imagery and 0.5 minutes of quiet imagery) and a 1-minute quiet recovery. During baseline, participants were instructed to stay still in the scanner without engaging in any mental activity. Before and after each trial, anxiety and alcohol craving ratings were elicited verbally for each using a 10-point Likert scale (0 = not at all, 10 = extremely high). Between each trial, participants were engaged in 2-minute progressive relaxation to normalize any residual anxiety or craving from the prior trial. This technique was mainly focused on relaxing physiological muscle tension and did not involve mental relaxation or imagery. After relaxation, anxiety and craving ratings returned to baseline, and there were no baseline differences in these ratings across trials.

Statistical Analysis

Functional MRI data were converted from Digital Imaging and Communication in Medicine format to analyze format using XMedCon.[24] To achieve steady-state equilibrium between

radiofrequency pulsing and relaxation, the first 10 images of each trial were discarded. Images were slice-time corrected using a custom-designed MATLAB program. Motion correction was implemented using Statistical Parametric Mapping version 5 for 3 translational and 3 rotational directions,[25] removing trials with linear motion greater than 1.5 mm and a rotation exceeding 2° (removed trials: 45 AD = 15 of 270 trials; 30 HC = 10 of 180 trials; and 30 AD = 11 of 180 trials). The recovery period (1 minute) was excluded from the data analysis to prevent carryover effects from the imagery period.

Individual-level analysis was conducted using a general linear model on each voxel in the entire brain volume with a task-specific regressor (2.5-minute imagery relative to a 1.5-minute baseline) using Yale BioImageSuite[26] (http://www.bioimagesuite.org/). To account for potential variability in baseline fMRI signal, drift correction was included in the general linear model; drift regressors were used to remove the mean time course, linear trend, quadratic trend, and cubic trend for each run. Each trial was normalized against the immediate baseline period preceding the script and then 2 trials of the same type were averaged. Functional images were spatially smoothed with a 6-mm Gaussian kernel, resulting in normalized beta maps in the acquired space (3.44 mm × 3.44 mm × 4 mm). To adjust for individual anatomical differences, 3 registrations were performed within the Yale BioImageSuite; a linear individual registration of raw functional image into a 2-dimensional anatomical image, 2-dimensional to 3-dimensional (1 mm × 1 mm × 1 mm) linear registration, and a nonlinear registration to reference 3-dimensional image, which is the Colin Brain[27] in Montreal Neurological Institute space. Then the output maps were converted to Analysis of Functional NeuroImages (AFNI) format for a group-level analysis.

Group analysis was conducted with AFNI[28] (http://afni.nimh.nih.gov). To examine the relationship between task-related brain activity and craving/relapse, whole-brain correlation analyses with alcohol craving and number of days of alcohol use during the 90-day follow-up were conducted using BioImageSuite. A familywise error rate correction was applied to correct for multiple comparisons using AFNI AlphaSim via Monte Carlo Simulation.[28,29] To examine the influence of outliers in the associations, Cook distance was used (score>1).

Additionally, Cox proportional hazard regression[30] was performed to investigate whether craving-correlated regions predicted the time to first alcohol relapse and time to heavy drinking relapse. Beta values of clusters identified in whole-brain correlation analyses were examined for their prediction of subsequent time to relapse in Cox regression using the multiple regression method. Preliminary analyses evaluated the contribution of sociodemographic characteristics (eg, age, sex, and race/ethnicity) and clinical variables (eg, nicotine smoking, years of alcohol use, baseline level of alcohol use before inpatient treatment, and history of mood and anxiety disorders) and recent other substance use to examine their independent effects on relapse risk. If any of these variables were associated with relapse outcome, they were included in the Cox regression models to evaluate their specific and independent effects.

When comparing 30 AD patients with 30 HC subjects, a random mixed-effects analysis was conducted with group (ie, patients and control subjects) as the between-subject fixed-effect factor, condition as the within-subject fixed-effect factor, and subject as the random-effect factor. Smoking status was included as a covariate. Two-tailed *t* tests were conducted on the significant effects for further comparisons. To correct for multiple comparisons, a familywise error correction was applied using AlphaSim in AFNI.[28]

Follow-up Interviews

After discharge from inpatient treatment, all patients were followed up with face-to-face interviews conducted at days 14, 30, and 90 postdischarge to evaluate relapse outcomes using urine and breathalyzer samples and the Form 90 substance use calendar[31] based on the timeline follow-back method,[32] as reported in our previous studies[6] (eAppendix in Supplement).

Results

Prediction of Alcohol Craving and Relapse Risk

Behavioral and Physiological Responses in 45 Alcohol-Dependent Patients

Linear mixed models were used with condition (stress, alcohol cue, and neutral) as within-subject factors. Replicating previous results,[6,12,33] we found that anxiety ratings ($F_{2,88}$ = 32.86, P < .001) were significantly higher in the stress (P < .001) and alcohol cue (P < .001) trials relative to the relaxed trials, and in stress relative to the alcohol cue trials (P < .01). Alcohol craving ratings ($F_{2,88}$ = 26.2, P < .001) were significantly greater in the stress (P < .001) and alcohol cue (P < .001) trials relative to the relaxed trials, as well as in the alcohol cue relative to the stress trials (P < .05). Averaged heart rate responses ($F_{2,85}$ = 17.22, P < .001) were significantly greater during the stress (P < .001) and alcohol cue (P < .001) trials relative to the relaxed trials, with no differences between stress and alcohol cue trials (Figure 1B, eFigure 1 in Supplement).

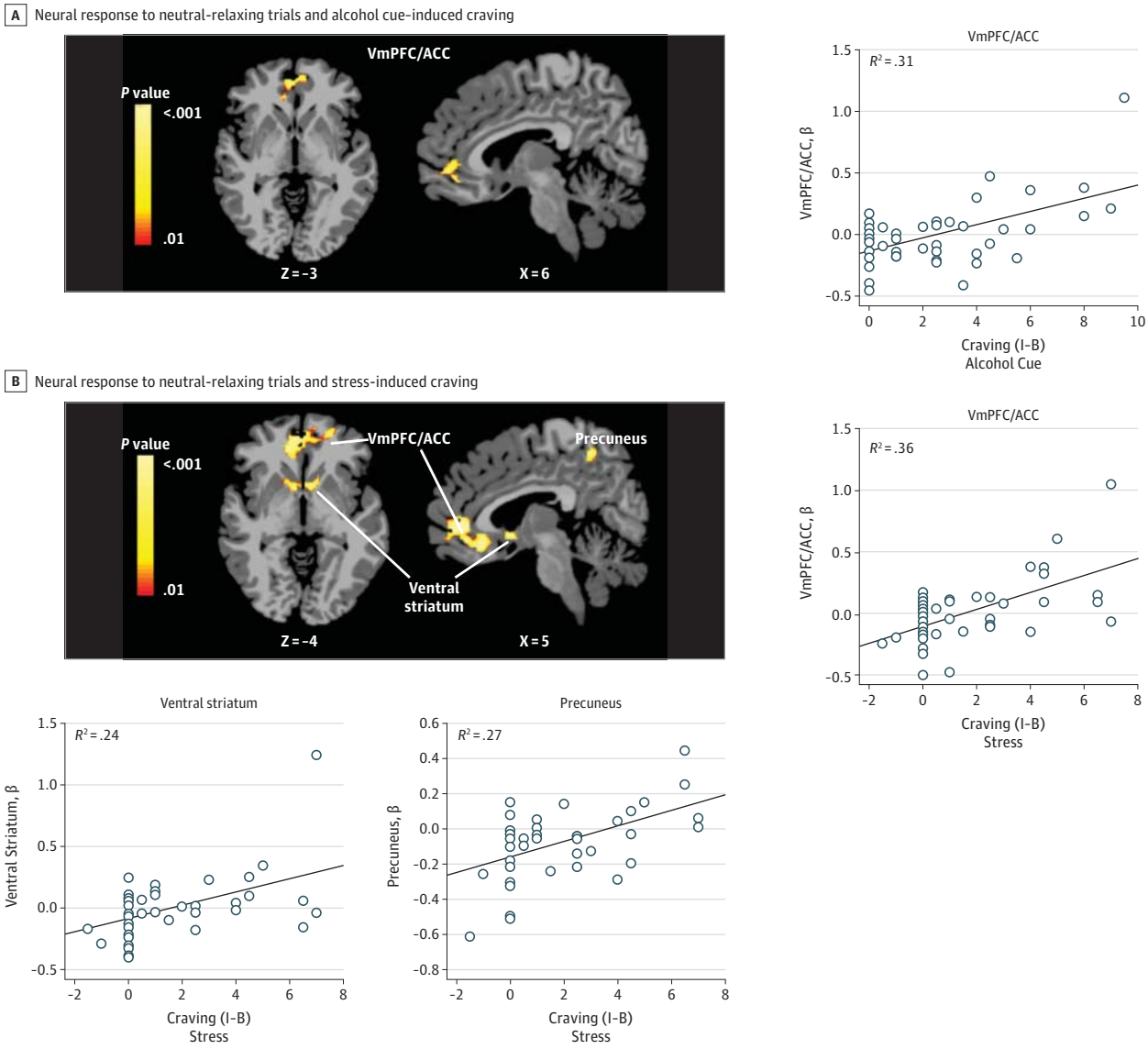
Neural Responses in Abstinent, Alcohol-Dependent Patients

Whole-brain analysis indicated that AD individuals showed increased activation in the corticolimbic-striatal regions during stress and alcohol cue exposure relative to neutral-relaxing imagery, including the medial prefrontal cortex (PFC), anterior cingulate cortex (ACC) and posterior cingulate cortex (PCC), superior frontal gyrus (SFG)/middle frontal gyrus (MFG), hippocampus, caudate, amygdala, temporal lobe, and cerebellum (P < .05, whole-brain corrected; eFigure 2 in Supplement).

Neural Correlates of Craving and Subsequent Time to Alcohol Relapse

Neural Correlates of Alcohol Craving | Higher alcohol cue-induced craving was significantly associated with hyperactivity in the ventromedial PFC (vmPFC) and ACC regions during neutral-relaxing trials (r = 0.56; R^2 = 0.31; **Figure 2**A). In addition, higher stress-induced craving was associated with hyperactivity in the vmPFC/ACC (r = 0.6; R^2 = 0.36), ventral stria-

Figure 2. Neural Correlates of Alcohol Craving in the Relapse Sample (N = 45)



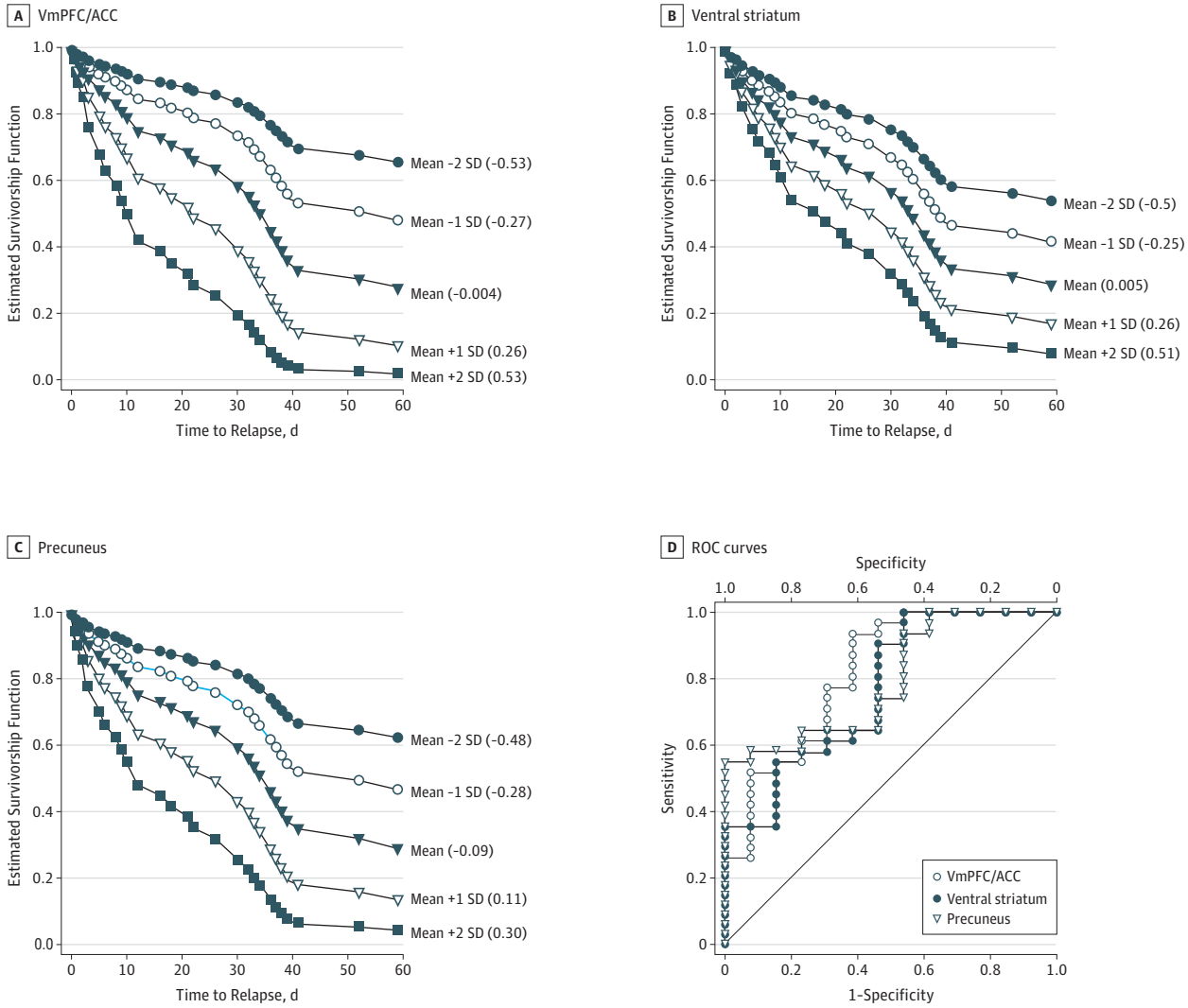
During neutral-relaxing trials, ventromedial prefrontal cortex (vmPFC)/anterior cingulate cortex (ACC) hyperactivity was significantly associated with alcohol craving during alcohol cue (A) and stress (B) conditions ($P < .01$, whole-brain corrected). In addition, hyperactivity in the ventral striatum and precuneus during the neutral condition was correlated only with stress-induced alcohol craving. There were no outliers in the associations between alcohol craving and brain activity in these regions during neutral-relaxing trials. There were no brain responses during stress and alcohol cue trials that significantly correlated with alcohol craving that survived whole-brain correction for multiple comparisons at $P < .01$. Yellow/red voxels = positive correlation. I − B indicates imagery minus baseline rating.

tum ($r = 0.49$; $R^2 = 0.24$), and precuneus ($r = 0.52$; $R^2 = 0.27$) during neutral-relaxed trials (Figure 2B). Furthermore, such neutral-relaxing state hyperactivity also contributed to a blunted vmPFC/ACC response in both the stress relative to neutral-relaxing and the alcohol cue relative to neutral relaxing contrasts correlating with alcohol craving in the respective conditions ($P < .01$, eFigure 3 in Supplement). There were no direct associations between alcohol craving and brain activity during stress and alcohol cue exposure that survived whole-brain correction for multiple comparisons at $P < .01$.

Relapse Rates | Of the 45 individuals, 44 patients were successfully followed up during the 90-day period. Alcohol use data collected from the timeline follow-back Substance Use Calendar, urine and breath test results, and collateral information indicated that relapse rates were 29.5% (13 of 44) at day 14, 45.5% (20 of 44) at day 30, and 70.5% (31 of 44) at day 90.

Prediction of Relapse Risk | Because stress-induced and alcohol cue–induced craving responses are predictive of relapse risk,[3,6,34] Cox proportional hazard regressions were con-

Figure 3. Estimated Survival Functions and Receiver Operating Characteristic (ROC) Curves for Craving-Related Neural Responses Predicting Subsequent Time to Alcohol Relapse



Estimated survival functions for time to first alcohol relapse (with the number of years of alcohol use and stress-induced alcohol craving held constant) is predicted by mean β value and +1 and +2 standard deviation (SD) above the mean and −1 and −2 SD below the mean in the ventromedial prefrontal cortex (vmPFC)/anterior cingulate cortex (ACC) (A), ventral striatum (B), and precuneus (C) during neutral-relaxing trials. The survival analysis is based on a 90-day period, but these figures of estimated survival functions show the x-axis up to day 60 because patients with altered brain activity 2 SD above the mean had relapsed by day 60. D, ROC curves for the vmPFC/ACC (area under the curve = 0.82; $\chi^2 = 4.94$; $P < .05$; 95% CI, 0.67-0.97), ventral striatum (area under the curve = 0.77; $\chi^2 = 1.96$; $P = .16$; 95% CI, 0.61-0.93), and precuneus (area under the curve = 0.79; $\chi^2 = 4.16$; $P < .05$; 95% CI, 0.66-0.93).

ducted to examine whether craving-related neural responses in the vmPFC/ACC, ventral striatum, and precuneus during neutral-relaxing states were predictive of subsequent time to first drink and time to heavy drinking relapse after accounting for the role of any significant demographic, clinical, and alcohol use history variables on relapse risk (**Figure 3, Table 1**). Of these variables, greater years of alcohol use (first relapse: $\chi^2 = 5.99$; $P < .01$; hazard ratio [HR], 1.06; 95% CI, 1.0-1.1; and heavy drinking relapse: $\chi^2 = 5.85$; $P < .02$; HR, 1.06; 95% CI, 1.0-1.1) and higher stress-induced alcohol craving (first relapse: $\chi^2 = 3.91$; $P < .05$; HR, 1.18; 95% CI, 1.0-1.4; and heavy drinking relapse: $\chi^2 = 3.13$; $P < .08$; HR, 1.15; 95% CI, 1.0-1.3) were each predictive of a shorter time to relapse; thus, they were included as covariates in the Cox models. No other clinical and behavioral measures were significantly predictive of alcohol relapse risk.

Table 1 shows the specific unique contribution of craving-related neural activation during the neutral-relaxing state to relapse risk. Hyperactivation in the vmPFC/ACC, ventral striatum, and precuneus during neutral-relaxing trials remained significantly predictive of a shorter time to subsequent relapse as well as relapse to heavy drinking in AD patients, even after accounting for stress-induced alcohol craving and years of alcohol use. Hyperactivity in the vmPFC/ACC during the neutral condition increased the likelihood of early relapse by 8.5 times and relapse to heavy drinking by 8.7 times. Hyperactivity in the

Table 1. Cox Regression Models of Neural Responses Predicting Time to Subsequent Relapse After Treatment Discharge[a,b]

		First Relapse			Relapse to Heavy Drinking		
Variable	Condition	χ^2	*P* Value	HR (95% CI)	χ^2	*P* Value	HR (95% CI)
VmPFC/ACC							
Craving (stress)		0.024	.88	0.98 (0.8-1.2)	0.01	.94	0.99 (0.8-1.2)
Duration of alcohol use, y[c]		6.1	.01	1.06 (1.0-1.1)	5.3	.02	1.06 (1.0-1.1)
VmPFC/ACC	Neutral	6.39	.01	8.45 (1.6-44.2)	7.39	.007	8.68 (1.8-41.2)
Ventral striatum							
Craving (stress)		0.58	.45	1.07 (0.9-1.3)	0.26	.61	1.05 (0.9-1.2)
Duration of alcohol use, y[c]		4.0	.05	1.05 (1.0-1.1)	3.58	.06	1.04 (1.0-1.1)
Ventral striatum	Neutral	3.5	.06	4.05 (0.9-17.5)	5.29	.02	5.68 (1.3-25.0)
Precuneus							
Craving (stress)		0.33	.57	1.05 (0.9-1.3)	0.18	.67	1.04 (0.9-1.2)
Duration of alcohol use, y[c]		4.11	.04	1.05 (1.0-1.1)	3.43	.06	1.04 (1.0-1.1)
Precuneus	Neutral	4.46	.03	11.96 (1.2-119.7)	4.64	.03	13.95 (1.3-153.5)

Abbreviations: ACC, anterior cingulate cortex; HR, hazard ratio; vmPFC, ventromedial prefrontal cortex.

[a] Includes stress-induced craving and years of alcohol use as covariates.

[b] A region of interest in the vmPFC/ACC regions was selected from overlapping vmPFC/ACC correlates between alcohol cue–induced craving (Figure 2A) and stress-induced craving (Figure 2B). Table 1 shows the relative increases in HRs after accounting for the effects of craving and years of alcohol use on time to first relapse and time to heavy drinking relapse in separate Cox proportional hazards regression models for each region. Hyperactivity in the vmPFC/ACC, ventral striatum, and precuneus during neutral-relaxing trials increased the prediction of earlier time to relapse substantially more than that seen by behavioral measures of craving and alcohol use history. For example, the vmPFC response substantially improved prediction of earlier first relapse by more than 8 times, the ventral striatum by more than 4 times, and the precuneus by more than 11 times. Stress-induced craving and duration of alcohol use were predictive of shorter time to alcohol relapse, thus they were included as covariates in the Cox models.

[c] Years of alcohol used throughout lifetime.

ventral striatum during neutral-relaxing trials increased the likelihood of early relapse by 4 times and relapse to heavy relapse by 5.7 times. Likewise, hyperactivity of the precuneus during neutral-relaxing trials increased the likelihood of earlier time to relapse by 12 times and 14 times for relapse to heavy drinking (Figure 3A-C). Similarly, as expected, stress-induced and alcohol cue–induced craving correlations with blunted vmPFC/ACC response in stress relative to neutral-relaxing and the alcohol cue relative to neutral-relaxing contrasts were also predictive of time to alcohol relapse and heavy drinking relapse (eFigure 3A and B in Supplement). Finally, we conducted receiver operating characteristic analysis to assess the predictive test characteristics of brain activity in the vmPFC/ACC, ventral striatum, and precuneus in the neutral condition. As shown in Figure 3D, the vmPFC/ACC region produced the most significant and accurate classification of relapsers vs nonrelapsers, covering above 0.8 of the area under the receiver operating characteristic (also see eTable 3 in Supplement for sensitivity, specificity, and +/− likelihood ratios).

Neural Correlates of Relapse Severity | Neural correlates of severity of alcohol use after relapse were assessed with whole-brain correlations between brain responses to stress, alcohol cue, and neutral-relaxed trials, as well as subsequent number of days of alcohol used during the 90-day follow-up period ($P < .01$, whole-brain corrected). Findings indicated that hyperactivation of the vmPFC/ACC and ventral striatum during neutral-relaxed trials, and hypoactivation in the vmPFC/ACC, precuneus, and the right side of the insula, superior/middle temporal gyrus, and lateral PFC during stress was associated with a greater number of days of alcohol used during follow-up (**Figure 4**, **Table 2**).

Group Differences Between Alcohol-Dependent Patients and Healthy Control Subjects

To examine whether patterns of the medial prefrontal responses associated with alcohol craving and relapse in the recovering AD patients was different from the response in demographically matched HC subjects, we compared 30 abstinent AD patients with 30 age-matched, sex-matched, and intelligence-matched HC subjects (eTable 1 in Supplement). Smoking status was included as a covariate.

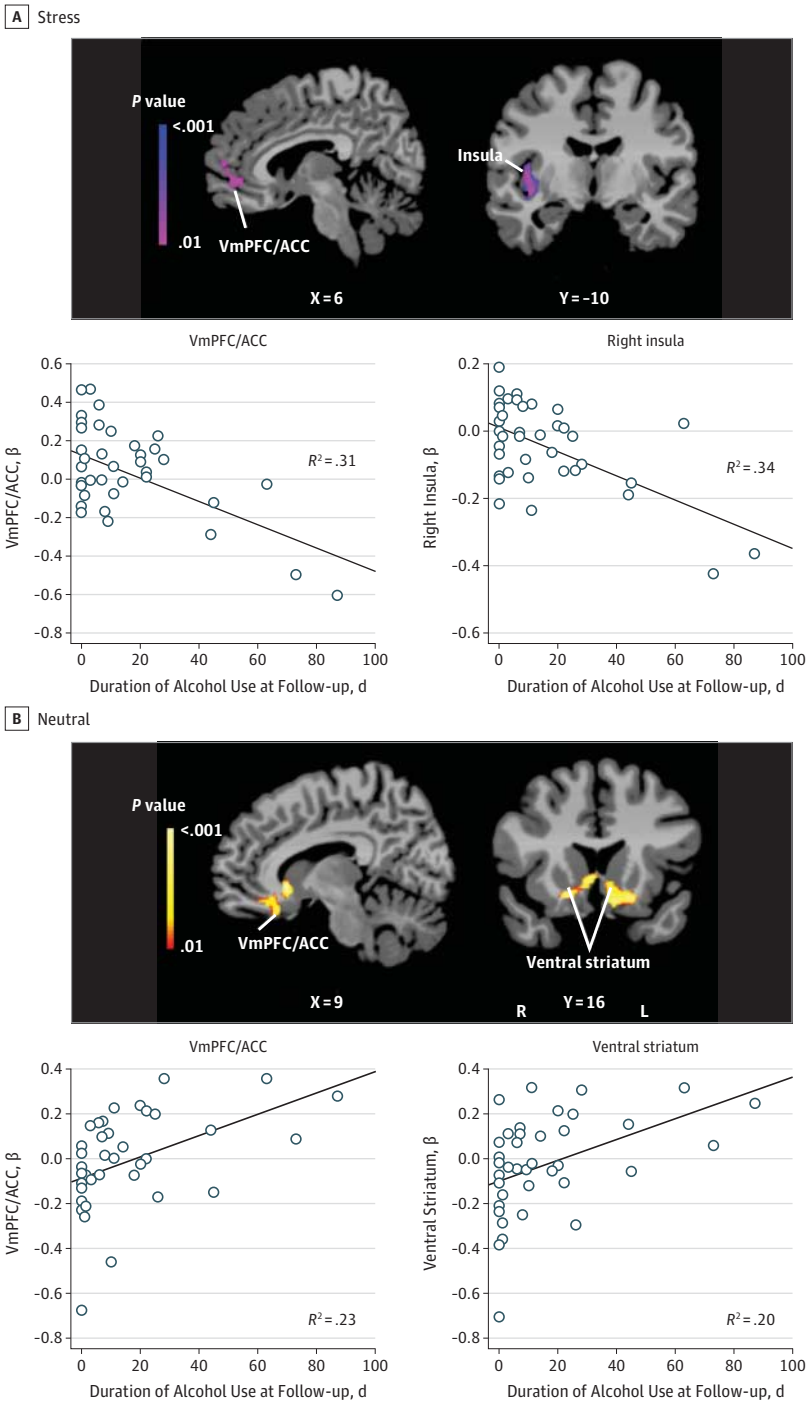
Differences in Behavioral and Physiological Responses

Consistent with previous research,[12] a significant effect of condition ($F_{2,116} = 42.1$, $P < .001$) and group × condition ($F_{2,116} = 6.0$, $P < .01$) interaction was observed for anxiety, with significantly higher anxiety during stress relative to neutral ($P < .001$) and to alcohol cue ($P < .001$) conditions, and higher anxiety during alcohol cue than in the neutral ($P < .001$) trials. Alcohol-dependent patients showed greater anxiety in the alcohol cue condition ($P < .01$), but not in the stress and neutral conditions relative to HC subjects (**Figure 5**A).

A significant effect of condition ($F_{2,116} = 19.3$, $P < .001$), group ($F_{1,58} = 10.2$, $P < .01$), and group × condition ($F_{2,116} = 8.0$, $P < .001$) was observed for alcohol craving, with significantly higher craving during alcohol cue than in neutral ($P < .001$) and stress ($P < .01$) conditions, and higher craving during stress than in the neutral-relaxing condition ($P < .001$). Alcohol-dependent patients showed significantly higher levels of craving in both alcohol cue ($P < .001$) and stress ($P < .01$) conditions relative to HC subjects (Figure 5B).

For heart rate (Figure 5C), significant effect of condition ($F_{1,109} = 9.3$, $P < .001$) indicated increased heart rate responses in both alcohol cue ($P < .01$) and stress ($P < .001$)

Figure 4. Neural Responses and Association With the Number of Days Alcohol Was Used After Relapse During Follow-up in 45 Alcohol-Dependent Patients



Whole-brain voxel-based correlation analysis ($P < .01$, whole-brain corrected) indicated reduced activation during the stress condition in the ventromedial prefrontal cortex (vmPFC)/anterior cingulate cortex (ACC) ($r = -0.56$, $R^2 = 0.31$) and right insula ($r = -0.59$, $R^2 = 0.34$) (A), as well as hyperactivation in the vmPFC/ACC ($r = 0.47$, $R^2 = 0.23$) and ventral striatum ($r = 0.45$, $R^2 = 0.20$) (B) during neutral-relaxed trials were each predictive of the number of days alcohol was used during the 90-day follow-up period. Brain response to alcohol cue trials was not predictive of the days alcohol was used during follow-up. Blue/purple voxels indicate negative association and yellow/red voxels indicate positive correlation. Coordinates are given in Montreal Neurological Institute space. There were no outliers in the relation between brain activity and the number of days alcohol was used during follow-up in the stress condition. In the neutral condition, there was 1 outlier (greater than 1 using Cook distance) in the relation between brain activity and the number of days alcohol was used. The correlation still remained significant even after removing this value. To reduce the influence of this value, the results here are presented after Winsorization and in Table 2 for the neutral condition. L indicates left; R, right.

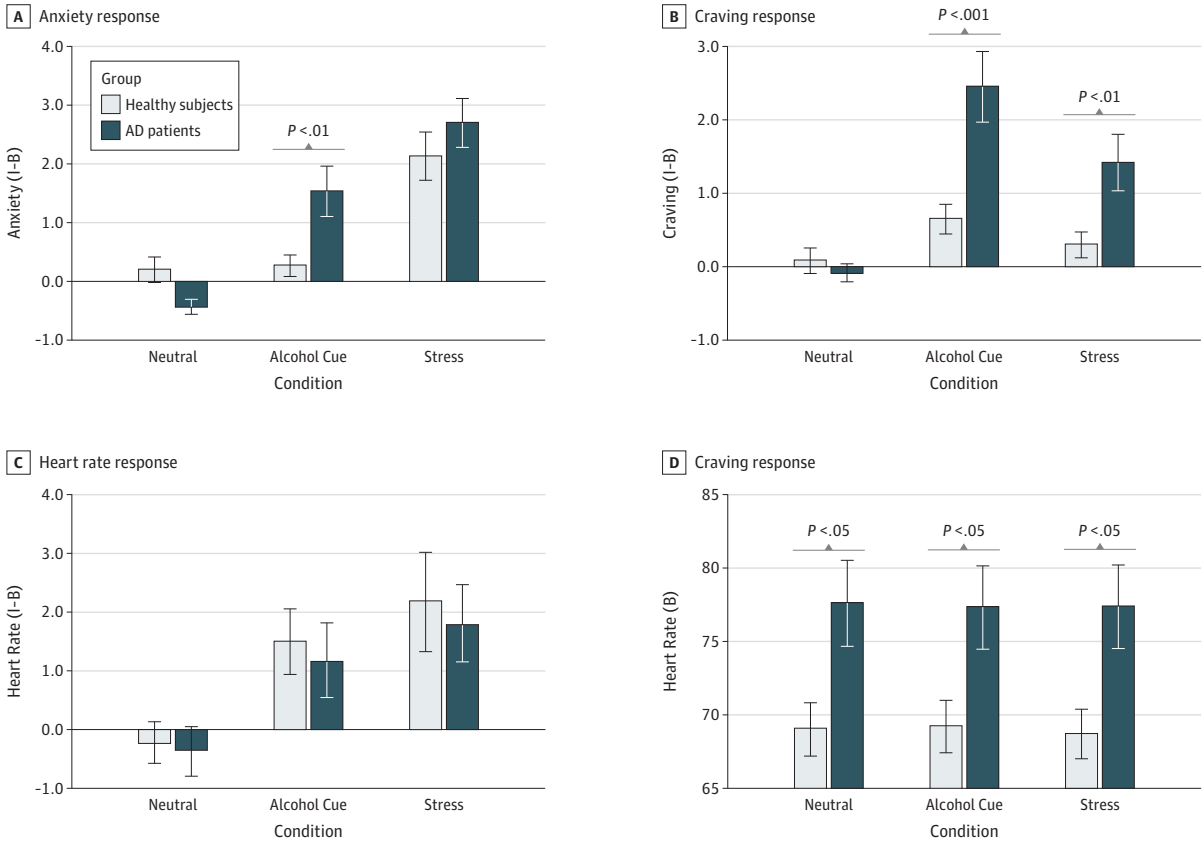
Table 2. Prediction of the Number of Days Alcohol Was Used in the 90-Day Follow-up Period[a]

Neural Correlates	Laterality	Brodmann Area	Coordinates			Volume, mm³	R^2	r
			X	Y	Z			
Neutral								
VmPFC/ACC	B	11, 32, 25	0	23	−13	3219	0.23	0.47
Ventral striatum	B		−6	14	−10	2248	0.20	0.45
Stress								
VmPFC/ACC	B	10, 11, 32	0	49	−3	1981	0.31	−0.56
Ventrolateral PFC	R	45, 46, 47	52	29	10	1438	0.30	−0.55
Posterior insula	R	13	41	−10	2	1490	0.34	−0.59
Superior/middle TG	R	21, 22	56	−26	−3	4408	0.34	−0.58
PCC/precuneus	B	23, 31, 7	2	−58	18	3375	0.33	−0.57

Abbreviations: ACC, anterior cingulate cortex; B, bilateral; PCC, posterior cingulate cortex; PFC, prefrontal cortex; R, right; TG, temporal gyrus; vmPFC, ventromedial prefrontal cortex.

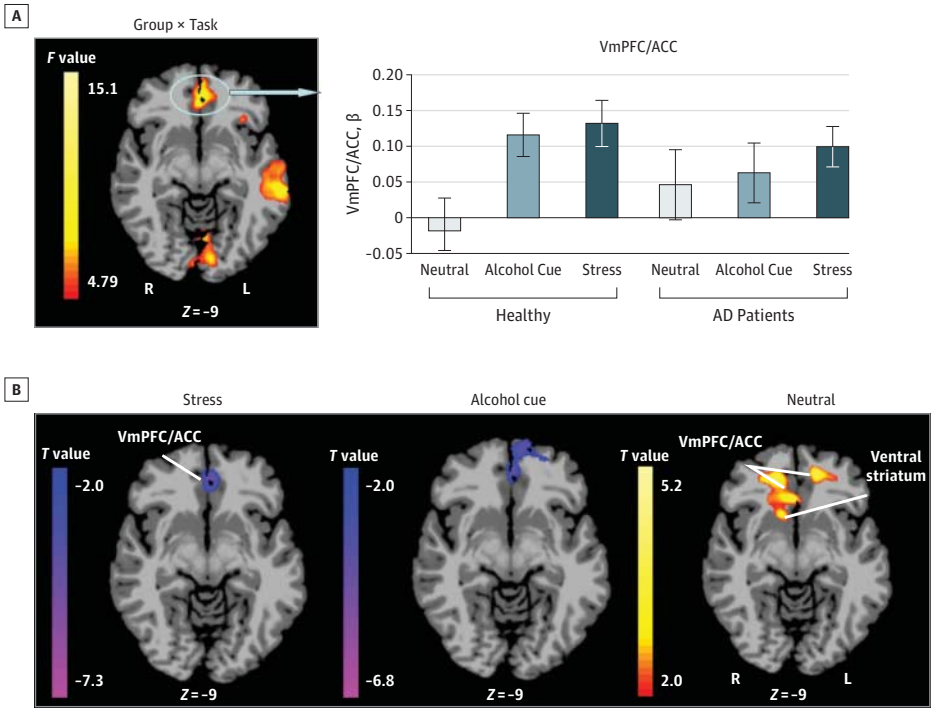
[a]Significant correlations at $P < .01$ (2 tailed, whole-brain familywise error corrected). Graphical representations of activity in these correlates are also provided in Figure 4. No associations were found between brain activity in the alcohol cue condition and follow-up days of alcohol used that survived correction for multiple comparisons.

Figure 5. Group Comparisons of the Means and Standard Errors of the Mean for Group Differences



Group comparisons of the means and standard errors of the mean for group differences (30 alcohol-dependent [AD] and 30 healthy control participants) in subjective anxiety (A), alcohol craving (B), heart rate response (C), and basal heart rate (D) during stress, alcohol cue, and neutral imagery trials. Baseline (B) ratings of anxiety and alcohol craving were not different between groups and in each condition. However, to account for any variation in B levels, all data analyses used change from baseline (imagery minus baseline [I − B]) values. A, Alcohol-dependent patients showed greater anxiety ratings during alcohol exposure ($t = 2.8$, $P < .01$), but not in the stress and neutral conditions relative to healthy control subjects. B, In addition, AD patients showed significantly higher levels of craving in both alcohol cue ($t = 4.42$, $P < .001$) and stress ($t = 2.7$, $P < .01$) exposures compared with healthy control subjects. C, For heart rate response, there is no group difference between AD and healthy control participants in each condition. D, However, in basal heart rate, there was a significant group main effect ($F_{1,56} = 5.92$, $P = .02$), such that AD patients displayed significantly greater basal heart rate than healthy control subjects ($t = 2.43$, $P < .05$).

Figure 6. Whole-Brain Voxel-Based Functional Magnetic Resonance Images Showing Group × Condition Interaction for 30 Alcohol-Dependent (AD) and 30 Healthy Control Subjects



A, Brain activity from group × condition interaction is displayed on the left, and mean β values in the ventromedial prefrontal cortex (vmPFC)/anterior cingulate cortex (ACC) in each condition per group are displayed in the bar graph ($P < .01$, whole-brain corrected). B, Group difference in the vmPFC/ACC during stress, alcohol cue, and neutral-relaxing conditions ($P < .05$, whole-brain corrected). Alcohol-dependent patients showed decreased activity in the vmPFC/ACC during the stress and alcohol cue conditions, but increased activity in the neutral-relaxing condition relative to healthy control subjects. Activity in the vmPFC/ACC during the neutral-relaxing condition was part of a significant cluster that included the ventral striatum, but the ventral striatum was not included in the significant vmPFC cluster for the stress or the alcohol cue conditions in AD patients relative to control subjects. Additional data from whole-brain contrasts between groups are shown in eTable 4 in Supplement. Coordinates are given in Montreal Neurological Institute space. L indicates left; R, right.

conditions relative to the neutral-relaxing condition. No other group or group × condition effects were significant. However, group differences in basal heart rate (group main effect: $F_{1,56} = 5.92$, $P = .02$) were observed, indicating significantly higher basal heart rate in AD patients compared with control subjects ($P < .05$) (Figure 5D).

Group Comparison of Neural Responses

Functional MRI results indicated that AD patients displayed altered vmPFC/ACC activation with vmPFC/ACC hypoactivity during stress and alcohol cue trials, but hyperactivity in a cluster connecting the vmPFC/ACC and ventral striatum during neutral-relaxed trials compared with control subjects (**Figure 6**; $P < .01$, whole-brain corrected).

Results of the whole-brain contrasts between groups are shown in eTable 4 in Supplement, along with group × condition interactions. In addition to the vmPFC/ACC differences between groups during the stress, alcohol cue, and neutral-relaxing conditions, we also found that selected areas of the dorsomedial PFC, left lateral orbitofrontal cortex/anterior insula, MFG, midbrain, superior/middle temporal lobe, PCC/precuneus, and cerebellum were hypoactive in AD patients compared with control subjects in the stress and alcohol cue conditions. During the neutral-relaxing condition, activity in the PCC/precuneus, lingual gyrus, and cerebellum was additionally increased in AD patients, while the left lateral orbitofrontal cortex and MFG and superior/middle temporal lobe were hypoactive compared with control subjects.

Discussion

The present study demonstrated that heightened activity in the vmPFC/ACC and ventral striatum during neutral-relaxing trials was associated with high stress-induced and cue-induced alcohol craving and alcohol relapse outcomes (relapse to first drink and to heavy drinking) in abstinent AD patients. Additionally, hypoactivity in selective brain regions involving the vmPFC/ACC, insula, and precuneus during stress exposure predicted alcohol use severity after relapse during the 90-day follow-up period. Furthermore, vmPFC/ACC activity during the neutral-relaxed state was significantly higher than the response in HC subjects, who showed deactivation of this region in the neutral-relaxed condition and robust vmPFC/ACC activation during stress and alcohol cue provocation conditions. This pattern of neural responses indicated

that vmPFC/ACC function in relaxed, stress, and alcohol cue contexts is disrupted during early recovery from alcoholism, and that such disrupted function contributes to high alcohol craving and relapse risk, thereby playing a significant role in jeopardizing recovery from alcoholism.

Relaxed-state hyperactivity in the vmPFC/ACC, ventral striatum, and precuneus observed in the current study is consistent with our previous neuroendocrine study showing greater adrenal sensitivity during the neutral-relaxing condition associated with shorter time to relapse in AD patients.[6] During neutral-relaxing trials, participants were guided through a personal relaxing situation, known to decrease anxiety and stress in control subjects and addicted patients, including AD individuals.[12,21] Neural hyperactivation in critical prefrontal-reward regions during this state is consistent with overactive physiological and neuroendocrine reactivity at basal levels in early recovering AD patients, including chronic alcohol-related upregulation of corticotropin-releasing hormone and noradrenergic signaling,[5,35,36] high basal cortisol levels,[7] heart rate,[21] disrupted heart rate variability and reduced inhibitory feedback control,[37] and neuronal hyperexcitability in the frontal region at resting state.[11] It is also well known that chronic alcohol use has a long-term excitatory effect on the central nervous system, leading to central nervous system hyperexcitability and gray matter volume reductions associated with multiple detoxifications and alcohol withdrawal symptoms, alcohol craving, and relapse risk.[2,38,39] Combined with the current data, it appears that the relaxed state was sensitive for detecting such alcohol-related hyperactivity in specific regions of the vmPFC/ACC, ventral striatum, and precuneus, suggesting the difficulty of AD patients in deactivating and relaxing this key circuitry involved in reward, craving, and impulse control. Interestingly, recent neuroimaging evidence on the pharmacological effects of alcohol, including the intravenous injection of alcohol[40] and a taste of alcohol,[41] indicates direct activation of these regions in healthy individuals, suggesting that repeated, high levels of alcohol use could sensitize these regions, leading to their hyperexcitability detectable under active relaxed conditions.

More specifically, in the current study, the vmPFC/ACC consistently predicted alcohol craving, time to relapse, and relapse severity, suggesting that this region could serve as a neural marker of alcohol relapse risk. The vmPFC/ACC is a core regulatory region of emotion and reward learning.[42,43] Given its crucial role in emotion regulation, heightened vmPFC activity during neutral-relaxing states may disrupt effective reward and stress regulation required during stress and alcohol cue conditions. This is supported by the altered vmPFC pattern observed in the comparison of matched AD and HC participants. Our findings indicate robust activation of the vmPFC/ACC during stress and alcohol cue trials in control subjects, consistent with this region's role in emotional and reward signaling, cognitive control, and decision making.[42,44] However, recovering AD patients showed hyperactivity in the vmPFC/ACC during neutral-relaxed trials, but hypoactive responses to stress and alcohol cue exposure. Hypoactive vmPFC/ACC during emotional challenge has been associated with affect dysregulation[45] and decision-making problems in substance-abusing patients,[17] supporting the findings of decreased vmPFC regulation during emotionally challenging states in our AD sample. This altered vmPFC pattern could also reflect neuronal inflexibility in AD patients with difficulty in changing brain responses in the face of challenging external or internal contexts. A poorly responsive and inflexible vmPFC/ACC during challenge would be unable to keep in check high levels of provoked alcohol craving, increasing the risk for relapse as shown in our study. In support of this speculation, when we directly compared brain activity during stress and alcohol cue exposure with activity in neutral-relaxing trials (eFigure 3 in Supplement), blunted vmPFC/ACC activity in the alcohol cue-neutral and stress-neutral contrasts were also predictive of high craving and a shorter time to relapse; the smaller the vmPFC/ACC response between emotionally challenging vs neutral-relaxing states, the greater the likelihood of high craving and early relapse in AD patients, further suggestive of disrupted, inflexible vmPFC function in early recovery from alcoholism.

The vmPFC exerts regulatory control over the reward and emotion modulatory circuit and closely interacts with brain regions in this area.[42,46-48] The functional connectivity analysis (eFigure 4 in Supplement) during the neutral condition also showed close connections of the vmPFC with regions including the ventral striatum, precuneus, and insula. Ventral striatal activity has been associated with reward response, and altered ventral striatal activity has been associated with alcohol cue–related craving and relapse in AD patients.[49,50] The precuneus is involved in default mode, resting state activity and the regulation of arousal states.[51] Close interactions of the vmPFC with these regions have been reported,[46,48] and preclinical research has shown the involvement of the excitatory pathways between the medial PFC and ventral striatum in drug and cue-induced reinstatement.[46] Therefore, concurrent dysfunction in these areas along with the vmPFC indicates disrupted reward/emotion modulatory circuits in AD patients, potentially due to regulatory deficits in the vmPFC.

Independent whole-brain correlation analysis with severity of alcohol use (number of drinking days) during follow-up provided further corroboration of the previously mentioned findings. Notably, recovering AD patients with hypoactivity in the vmPFC/ACC and insula during stress trials, but hyperactivity in the VmPFC/ACC and ventral striatum in neutral-relaxed trials, used alcohol on a greater number of days after discharge. During stress exposure, additional reduced responses in the precuneus, and right lateral PFC and MTG were also associated with relapse severity. The vmPFC/ACC and insula are key regions involved in the neurovisceral integration and regulation of emotional arousal and stress,[43,52] especially as it pertains to self-control and decision making.[17] The involvement of the insula has been reported in nicotine craving, smoking cessation, and methamphetamine relapse.[53,54] In addition, previous research has reported gray matter volume reduction in a stress modulatory circuit in AD patients including the vmPFC,[39,55] ACC, insula,[56] and precuneus.[55] Hypoactivity in these regions during stress being predictive of greater number of days of alcohol use indicates that hypofunction or gray matter reduction in stress modulatory regions may contribute to the severity of alcohol relapse.

Taken together, the present findings indicate that a pattern of vmPFC/ACC hyperresponsiveness during neutral-relaxed states and hypoactivation during stress states may represent the functional neural state that drives high alcohol craving and relapse risk in recovering AD patients. Although hypoactive response patterns during stress and alcohol cue in our study are consistent with previous neuroendocrine findings,[6,8] they are inconsistent with previous alcohol cue challenge studies. Using different provocation paradigms (eg, visual alcohol cue stimuli), previous research has reported an increased response to alcohol-related visual stimuli in the medial PFC, ACC, striatum,[49] ventral striatum,[57] and PFC and anterior thalamus,[58] as well as increased prefrontal activity in response to alcohol-related words[59] or odors.[60] Visual alcohol cue–related response in the ventral striatum was also associated with relapse status.[61] While the foci of brain regions in the current study are similar to these studies, variations in activity are likely related to differences in experimental stimuli and study designs (externally vs internally focused stimuli) and in task specificity (eg, experiencing emotion via recall). On the other hand, multiple neuroimaging studies have found hypoactive vmPFC response to cognitive and emotionally challenging states associated with poor regulatory function and decision-making problems,[17,42,45,62] suggesting that decreased vmPFC/ACC response to alcohol cue/stress in our AD sample relative to control subjects could reflect their vulnerability to poor emotional control and decision-making impairments (eg, return to alcohol use).

The present findings have direct implications for clinical care. If current findings are validated in future studies, disrupted vmPFC/ACC function may be further developed as a neural marker to identify those who are most at risk for alcohol relapse. Specifically, the inclusion of relaxation trials in the current study allowed the identification of a hyperreactive neural resting/relaxed state in recovering AD patients, which modulated provoked alcohol craving during the experiment, as well as subsequent alcohol relapse outcomes. This suggests that future research exploring altered resting/relaxed states in AD patients may elucidate mechanisms underlying compromised cognitive and emotional regulatory capacity in recovering AD patients. Furthermore, medications and behavioral treatment strategies that restore prefrontal function could be of benefit to those who are at high risk for relapse. For example, α1-adrenergic antagonists, such as prazosin, are known to rescue prefrontal neurons from the deleterious effects of chronic stress[63] and decrease both chronic alcohol-related withdrawal symptoms and stress-induced reinstatement of alcohol seeking in animals.[64,65] Recent studies have also shown its benefit in decreasing stress- and cue-induced alcohol craving and alcohol use in humans.[66,67] Similarly, novel stress-related behavioral treatment strategies that restore vmPFC function by reducing prefrontal, relaxed-state hyperresponsiveness and normalizing stress state hypoactivity may also be of benefit in improving alcohol relapse outcomes.

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