ORIGINAL ARTICLE

Effect of Brain Structure, Brain Function, and Brain Connectivity on Relapse in Alcohol-Dependent Patients

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Context: In alcohol-dependent patients, brain atrophy and functional brain activation elicited by alcohol-associated stimuli may predict relapse. However, to date, the interaction between both factors has not been studied.

Objective: To determine whether results from structural and functional magnetic resonance imaging are associated with relapse in detoxified alcohol-dependent patients.

Design: A cue-reactivity functional magnetic resonance experiment with alcohol-associated and neutral stimuli. After a follow-up period of 3 months, the group of 46 detoxified alcohol-dependent patients was subdivided into 16 abstainers and 30 relapsers.

Setting: Faculty for Clinical Medicine Mannheim at the University of Heidelberg, Germany.

Participants: A total of 46 detoxified alcohol-dependent patients and 46 age- and sex-matched healthy control subjects

Main Outcome Measures: Local gray matter volume, local stimulus–related functional magnetic resonance imaging activation, joint analyses of structural and functional data with Biological Parametric Mapping, and connectivity analyses adopting the psychophysiological interaction approach.

Results: Subsequent relapsers showed pronounced atrophy in the bilateral orbitofrontal cortex and in the right medial prefrontal and anterior cingulate cortex, compared with healthy controls and patients who remained abstinent. The local gray matter volume–corrected brain response elicited by alcohol-associated vs neutral stimuli in the left medial prefrontal cortex was enhanced for subsequent relapsers, whereas abstainers displayed an increased neural response in the midbrain (the ventral tegmental area extending into the subthalamic nucleus) and ventral striatum. For alcohol-associated vs neutral stimuli in abstainers compared with relapsers, the analyses of the psychophysiological interaction showed a stronger functional connectivity between the midbrain and the left amygdala and between the midbrain and the left orbitofrontal cortex.

Conclusions: Subsequent relapsers displayed increased brain atrophy in brain areas associated with error monitoring and behavioral control. Correcting for gray matter reductions, we found that, in these patients, alcohol-related cues elicited increased activation in brain areas associated with attentional bias toward these cues and that, in patients who remained abstinent, increased activation and connectivity were observed in brain areas associated with processing of salient or aversive stimuli.

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To date, only a few neuroimaging studies[1,2] assessed factors that predict relapse in alcohol dependence. One major situation often provoking relapse is the confrontation with stimuli that have been regularly associated with alcohol consumption; owing to addiction-related learning processes, these formerly neutral stimuli can become conditioned cues and evoke alcohol craving or relapse even without the presence of alcohol itself.[3-9] Craving for alcohol is often denied by detoxified alcohol-dependent patients, although they still show high levels of relapse.[9] A study by Grüsser et al[10] revealed that addicted patients evaluated alcohol-related cues negatively, reported little craving, but still displayed a positive emotional response to alcohol cues as assessed by startle response inhibition. The assumption that alcohol cues can motivate alcohol intake even in the absence of conscious craving is in accordance with the hypothesis of Tiffany and Carter,[9] who suggested that habitual drug intake rather than craving plays a major role in the maintenance of drug addiction.

Imaging studies of alcohol-dependent patients identified brain areas activated by alcohol-associated vs neutral cues: the orbitofrontal cortex (OFC)[11,12]; the medial prefrontal cortex (MPFC) and the adja-

cent anterior cingulate cortex (ACC)[1,11,13,14]; and the ventral and central striatum, including the nucleus accumbens[12,15-18] and the basolateral amygdala.[19] Interestingly, increased brain responses in rats and humans elicited by alcohol-associated stimuli in different parts of the MPFC were associated with an increased prospective risk of relapse and a dysfunction of dopaminergic neurotransmission in the ventral striatum/nucleus accumbens.[1,5,20-22]

Brain function in alcohol-dependent patients may be affected by brain atrophy in cortical and subcortical regions, which has also been associated with the prospective relapse risk[23] (for review, see Sullivan[24]). Rando et al[25] demonstrated that gray matter volume deficits in medial frontal and posterior parietal-occipital brain regions are predictive of an earlier return to alcohol use in detoxified alcohol-dependent patients. Wrase et al[23] and Benegal et al[26] observed reduced volumes of the cingulate and parahippocampal gyri, as well as the amygdala, in subsequent relapsers and, interestingly, also in young, alcohol-naive subjects at high risk for alcohol dependence due to a positive family history. Furthermore, the latter group showed smaller volumes of the superior frontal gyrus, the thalamus, and the cerebellum than did individuals without a positive family history.[26] Recently, Durazzo et al[27] showed morphological abnormalities in relapsers vs abstainers in the right rostral middle and caudal middle frontal gyri and in the lateral OFC, bilaterally—regions involved in "top-down" regulation and modulation of internal drive states (ie, emotions, reward processing, and evaluation) and of behavior, which may increase risk for relapse. In addition, decreases in neuronal integrity as indicated by altered surrogate marker concentration (*N*-acetylaspartate and choline) in temporal gray matter and frontal white matter in relapsers compared with abstainers were shown.[28] Although there is strong evidence for pronounced cortical gray matter loss in addicted individuals, which most likely interferes with brain responses, to our knowledge, there is only 1 published study[29] that focuses on addiction and uses the information of both brain function and structure. Bustamante et al[29] observed reduced activation in brain areas related to the attention system (the dorsal part of the inferior parietal cortex) in cocaine-dependent men compared with healthy subjects during a verbal working memory task while controlling for individual differences in gray matter volume within regions of functional differences.[29] In our study, we combine structural and functional brain imaging to examine the brain regions involved in the response toward alcohol-associated cues as already described, with a focus on prospective relapse among detoxified alcohol-dependent patients. Specifically, we assume decreased gray matter volume in frontocortical and limbic areas in prospective relapsers and an increased brain response toward alcohol-associated cues in the MPFC in the same group. Moreover, we will compare standard functional imaging analyses with a new analytic approach using a local (voxelwise) correction for gray matter loss as additional information.

Chronic alcohol intake may disrupt not only local neuronal functioning but also the connectivity between different brain areas. A study[30] investigating functional connectivity during a backward masking paradigm with smoking-related stimuli in smokers and nonsmokers observed altered connectivity between the anterior cingulate cortex and the amygdala in smokers but not in nonsmokers. Gu et al[31] reported decreased functional connectivity within the mesocorticolimbic brain reward system in chronic cocaine users (1) between the ventral tegmental area (VTA) and a region encompassing the thalamus/lentiform nucleus/nucleus accumbens, (2) between the amygdala and the MPFC, and (3) between the hippocampus and the dorsal MPFC. Moreover, they showed that the strength of functional connectivity between the VTA and the thalamus/lentiform nucleus/nucleus accumbens was negatively correlated with years of cocaine use.[31] Based on these findings, we additionally performed an exploratory brain connectivity analyses to investigate how alcohol-associated cues vs neutral cues modulate functional connectivity in alcohol-dependent patients.

METHODS

SUBJECTS

A total of 46 abstinent alcohol-dependent patients and 46 healthy control subjects participated in our study. Groups were matched for age and sex (**Table 1**). Alcohol-dependent patients fulfilled the diagnostic criteria for alcohol dependence according to the *ICD-10* and the *DSM-IV*. A standardized clinical assessment using structured clinical interviews[32,33] was performed to exclude other Axis I psychiatric disorders in patients and control subjects and to exclude Axis II disorders in the latter group. The severity of alcoholism was assessed using the Alcohol Dependence Scale,[34] and the amount of lifetime alcohol intake was measured by applying the Lifetime Drinking History interview.[35] Severity of current alcohol craving was assessed with the Alcohol Craving Questionnaire.[36] Patients had no previous substance dependence or current substance abuse other than alcoholism and nicotine use (which was determined by random urine drug testing). The socioeconomic status of patients was measured using the Hollingshead Index of Social Position.[37] The severity of depression was assessed using the Hamilton Depression Rating Scale.[38] The volumes of erythrocytes (mean corpuscular volume), aspartate aminotransferase, and γ-glutamyl transpeptidase were also assessed as clinical markers. Patients with neurological or hepatic impairment (eg, liver cirrhosis) were excluded.

Sociodemographic and clinical data were analyzed with PASW Statistics 18 (SPSS Inc) using 2-sample *t* tests with exceptions for Hollingshead Index of Social Position (Mann-Whitney *U* test) and sex (χ^2 test). Missing data were replaced by the median value for interval-scaled data and by the modal value for ordinal- or nominal-scaled data. All results are listed in Table 1 and **Table 2**.

Alcohol-dependent patients had been detoxified on a ward. This detoxification was supported by medication in 40% of the subsequent relapsers and in 44% of the subsequent abstainers (no significant group differences; $P = .64$ determined by use of the Kruskal-Wallis test) (eAppendix, http://www.archgenpsychiatry.com). All patients were abstinent for at least 1 week (mean [SD] duration, 19.74 [22.66] days) before structural and functional magnetic resonance imaging (fMRI) and were free of benzodiazepine or chlormethiazole medication for at least 1 week (>4 half-lives). Current substance or alcohol abuse was checked by random breath and urine testing. After scanning, patients were probed during a 2-week cycle by one of the researchers (J.W.) for a follow-up period of 3 months. Alcohol consumption was recorded with the Form 90, a standard tool for retrospective assessment of alcohol intake.[39] The researcher (J.W.) was blinded to the imaging data during the postsession visits with the patients. In accordance with

Table 1. Clinical Data of the Group of Alcohol-Dependent Patients and the Group of Healthy Control Subjects

Characteristic	Healthy Controls[a] (n = 46) Mean (SD)	Amount of Missing Data[b]	Alcohol-Dependent Patients[a] (n = 46) Mean (SD)	Amount of Missing Data[b]	*P* Value
Age, y	39.37 (7.72)	0	40.37 (6.68)	0	.51
ADS score	1.02 (1.90)	4	16.43 (5.89)	4	<.001[c]
Lifetime alcohol intake,[d] kg	85.01 (104.18)	9	619.61 (497.64)	12	<.001[c]
Age at onset, y			29.02 (8.38)	2	
ACQ score	55.48 (9.32)	4	72.74 (26.60)	4	<.001[c]
No. of cigarettes per day	4.28 (8.49)	0	19.89 (13.75)	3	<.001[c]
HDRS score	1.30 (4.11)	5	3.37 (3.07)	3	.01[c]
Hollingshead ISP	5.50 (1.30)	5	4.76 (1.62)	3	.02[c]
Erythrocytes,[e] mm^3	91.05 (4.18)	9	98.01 (13.88)	8	.002[c]
AST, U/L	10.15 (2.08)	8	33.79 (36.48)	6	<.001[c]
G-GT, U/L	14.07 (7.88)	10	177.30 (264.34)	6	<.001[c]

Abbreviations: ACQ, Alcohol Craving Questionnaire; ADS, Alcohol Dependence Scale; AST, aspartate aminotransferase; G-GT, γ-glutamyltranspeptidase; HDRS, Hamilton Depression Rating Scale; ISP, Index of Social Position.

SI conversion factor: To convert AST to microkatals per liter, multiply by 0.0167.

[a] A total of 30 men and 16 women (in each group); *df* = 90.

[b] Missing data were replaced by the median value for interval-scaled data and by the modal value for ordinal- or nominal-scaled data.

[c] Using the *t* test, we found $P < .05$ for comparisons between healthy control subjects and alcohol-dependent patients.

[d] From the Lifetime Drinking History interview.

[e] Mean corpuscular volume.

Table 2. Clinical Data of the 2 Patient Groups

Characteristic	Relapsers[a] (n = 30) Mean (SD)	Amount of Missing Data[c]	Abstainers[b] (n = 16) Mean (SD)	Amount of Missing Data[c]	*P* Value
Age, y	39.80 (6.33)	0	41.44 (7.38)	0	.43
ADS score	16.58 (5.62)	3	16.16 (6.55)	1	.82
Lifetime alcohol intake,[d] kg	684.51 (536.04)	7	497.934 (403.99)	5	.23
Age at onset, y	27.77 (7.75)	2	31.38 (9.24)	0	.17
ACQ score	73.37 (29.45)	3	71.56 (21.06)	1	.83
No. of days abstinent prior to scanning	14.96 (11.80)	3	27.81 (32.96)	1	.15
No. of days until relapse	30.22 (20.02)	5			
Alcohol intake during follow-up, g	5806 (6288)	7			
No. of patients receiving detox medication		0		0	.64[e]
No. of cigarettes per day	21.30 (12.82)	3	17.25 (15.41)	0	.35
HDRS score	3.40 (3.36)	3	3.31 (2.52)	0	.93
Social status, Hollingshead ISP	5.13 (1.17)	3	4.06 (2.11)	0	.03[f]
Erythrocytes,[g] mm^3	97.20 (16.20)	5	99.52 (8.21)	3	.60
AST, U/L	36.83 (41.44)	4	28.00 (24.84)	2	.44
G-GT, U/L	136.47 (179.79)	4	253.88 (352.71)	2	.23
Sex		0		0	.76[h]

Abbreviations: ACQ, Alcohol Craving Questionnaire; ADS, Alcohol Dependence Scale; AST, aspartate aminotransferase; G-GT, γ-glutamyltranspeptidase; HDRS, Hamilton Depression Rating Scale; ISP, Index of Social Position.

SI conversion factor: To convert AST to microkatals per liter, multiply by 0.0167.

[a] A total of 19 men and 11 women; *df* = 44.

[b] A total of 11 men and 5 women; *df* = 44.

[c] Missing data were replaced by the median value for interval-scaled data and by the modal value for ordinal- or nominal-scaled data.

[d] From the Lifetime Drinking History interview.

[e] Kruskal-Wallis test.

[f] Using the *t* test, we found $P < .05$ for comparisons between relapsers and abstainers.

[g] Mean corpuscular volume.

[h] Fisher exact test.

standard clinical trials,[40,41] relapse was defined as a consumption of more than 60 g of alcohol in men or more than 40 g of alcohol in women during the assessment period (3 months). Random alcohol blood and breath tests were performed, levels of carbohydrate-deficient transferrin and γ-glutamyltransferase were evaluated, and relatives or members of their support group were contacted to verify the patient's statements. The patients were not provided follow-up treatment after detoxification during the period of MRI and relapse assessment. Our study was approved by the ethics committee of the Faculty for Clinical Medicine

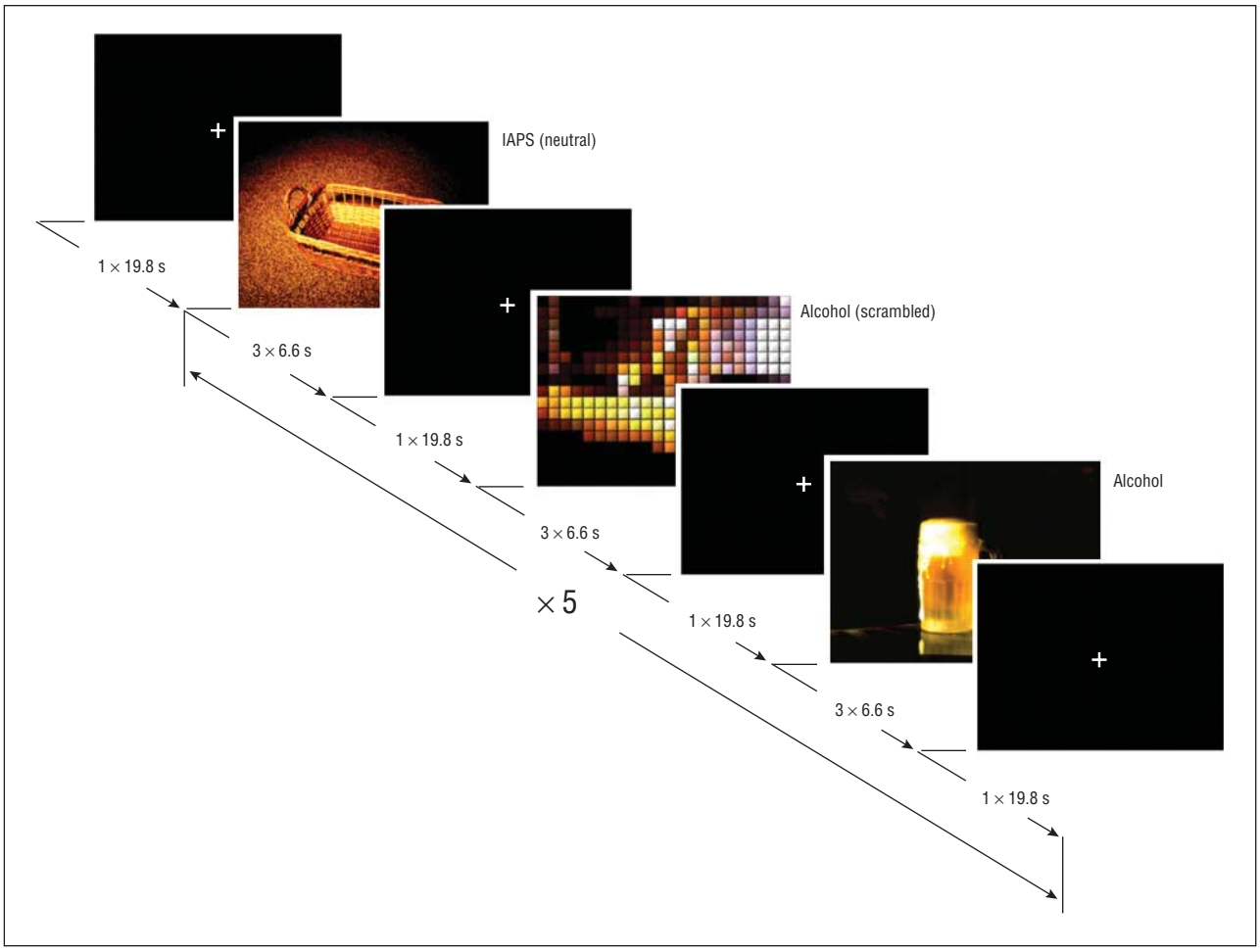

Figure 1. Alcohol-related pictures, their scrambled versions, and affectively neutral pictures of housekeeping items from the International Affective Picture System (IAPS),[42] serving as stimuli. The functional response elicited by alcohol-related pictures was compared with the response following neutral IAPS pictures.

Mannheim at the University of Heidelberg, Germany, and informed written consent was obtained from all participants.

After the follow-up period, the recorded data were analyzed with regard to the subsequent relapse, which divided the group of patients into 16 abstainers and 30 relapsers. Within the abstainers, there was only 1 patient who consumed 10 g of alcohol during the observation period, whereas the other abstainers did not drink any alcohol. In contrast, the smallest amount of alcohol consumed in the group of relapsers during the 3-month follow-up period was 207 g. Relapse occurred after a mean (SD) 30.2 (20.0) days.

STIMULATION PROTOCOL

A total of 15 alcohol-related pictures (CUE_{alc}) and 15 affectively neutral pictures ($IAPS_{neutral}$) of, for example, housekeeping items from the International Affective Picture System (IAPS),[42] as well as their scrambled versions, served as stimuli (**Figure 1**). In previous studies,[12,43] the use of CUE_{alc} elicited a reliable craving in detoxified alcohol-dependent patients. During fMRI scanning, the alcohol-associated and control stimuli were presented in a block design. The paradigm consisted of 5 stimulation blocks per category separated by a fixation condition (fixation cross). Three images per stimulation block were randomly presented for 6.6 seconds each. The stimulation, as well as fixation periods, had a total duration of 19.8 seconds. After scanning, patients were counseled if they experienced persistent craving for alcohol.

MRI SCANNING

The blood oxygen level–dependent (BOLD) fMRI experiment was conducted using a 1.5-T MR scanner (Magnetom VISION; Siemens) equipped with a standard circularly polarized head coil using a 2-dimensional gradient-echo echo-planar imaging pulse sequence. A total of 256 whole-brain functional images (repetition time, 3300 milliseconds; echo time, 66 milliseconds; flip angle, 90°; image matrix, 64 × 64; field of view, 220 × 220 mm^2; voxel size, 3.4 × 3.4 × 5.0 mm^3) comprising 24 slices approximately parallel to the bicommissural plane were collected per subject. The first 3 volumes of each session were discarded to allow for magnetic field stabilization. For anatomical reference and for brain morphometric analysis, a 3-dimensional magnetization-prepared rapid gradient echo image was acquired (repetition time, 11.4 milliseconds; echo time, 4.4 milliseconds; flip angle, 12°; image matrix, 256 × 256; field of view, 256 × 256 mm^2; 162 sagittal slices; voxel size, 1 × 1 × 1 mm^3).

DATA PROCESSING AND ANALYSIS

Data processing and analysis were performed with the Statistical Parametric Mapping 8 (SPM8) software package (http://www.fil.ion.ucl.ac.uk/spm/; Wellcome Department of Imaging Neuroscience), the ArtRepair software (http://cibsr.stanford.edu/tools/human-brain-project/artrepair-software.html) developed by Mazaika and colleagues,[44] the voxel-based morphometry toolbox for SPM version 8 (VBM8) developed by

Christian Gaser, PhD, and colleagues (http://dbm.neuro.uni-jena.de/vbm8/), and the Biological Parametric Mapping (BPM) toolbox (http://fmri.wfubmc.edu/cms/software) developed by Casanova and colleagues.[45] Corresponding brain regions were identified with reference to the Anatomy Toolbox for SPM version 1.7 as developed by Eickhoff et al.[46]

VOXEL-BASED MORPHOMETRY

The structural scan of each subject was denoised by applying an optimized blockwise nonlocal means filter algorithm as described by Coupe et al,[47] morphed into the standard space (diffeomorphic anatomical registration through exponentiated lie algebra [DARTEL] Montreal Neurological Institute [MNI] template of 550 healthy control subjects of the IXI [Information Extraction From Images] database) using the DARTEL normalization as implemented in SPM8,[48] and segmented into tissue classes using the adaptive maximum a posterior[49] and partial volume effect[50] techniques as implemented in the VBM8 toolbox. From these tissue segments, group-specific templates were created for gray and white matter, as well as cerebrospinal fluid, and spatially smoothed with an isotropic Gaussian kernel (full-width at half-maximum [FWHM] = 10 mm). In a second pass, the procedure was repeated using the group-specific templates. Finally, the affine and nonlinear intensity-modulated gray matter segments were spatially smoothed with a 3-dimensional isotropic Gaussian kernel (FWHM = 10 mm). A 1-way analysis of covariance was conducted for the smoothed gray matter segments with the between-subject factor group (controls, abstainers, and relapsers) and the individual intracranial volume (computed with the VBM8 toolbox).

BOLD fMRI

Echo-planar imagings were corrected for technical artifacts, such as signal-to-noise decrease in single slices, using the denoising function of the ArtRepair software. The ensuing SPM preprocessing consisted of motion correction, calculation of a mean echo-planar image, stereotactical normalization using the set of transformation parameters obtained from the corresponding structural image (which has been coregistered to the mean echo-planar imaging before), resampling to an isotropic voxel size of $3.5 \times 3.5 \times 3.5$ mm^3, and spatially smoothing by use of an isotropic kernel (FWHM = 8 mm). The resulting voxel time series were high-pass–filtered with a cutoff frequency of 128 seconds. Serial correlations from aliased cardiological and respiratory effects were accounted for using a first-order autoregressive model.

Statistical analysis was performed in a 2-stage mixed-effects model. In the first single-subject stage, neural activity was modeled by a boxcar function. The BOLD response was modeled by convolving these boxcar functions with the canonical hemodynamic response function as implemented in SPM. The resulting time courses were down-sampled for each scan to form the explanatory variables of a general linear model. The resulting general linear model comprised 2 regressors for the conditions of interest (CUE_{alc}, $IAPS_{neutral}$), one for the scrambled pictures, one for each of the 6 rigid-body movement parameters estimated during motion correction, and a single constant representing the mean over scans. Weighting parameters for the general linear model were estimated by use of a restricted maximum likelihood fit. Linear contrast images were computed for $CUE_{alc} > IAPS_{neutral}$ for each subject.

For these contrast images, second-level random-effects analyses were conducted with the standard SPM approach and with the BPM toolbox. Taking into account that VBM analysis revealed a massive but locally variable cortical atrophy in the group of alcohol-dependent patients, we used the individual local gray matter volume as a voxel-wise covariate in our BPM analyses. To assess the interaction between group and cue reactivity, we computed a 1-way analysis of covariance for repeated measures with "group" as a between-subject factor (controls, abstainers, and relapsers). To compare the groups, T contrasts were computed on the resulting parameter estimates.

The psychophysiological interaction (PPI) approach[51-53] was used to assess the functional coupling between different brain regions in relation to the experimental context (ie, the presentation of alcohol-related stimuli). The PPI is defined as the change in connectivity of one brain area (the so-called seed region) to another with the experimental or psychological context (ie, the alcohol vs the neutral stimuli).[52] In PPI analysis, whole-brain connectivity on a voxel-by-voxel basis between the time series of the seed region and the time series of all other voxels, modulated by the experimental stimulus, is computed. Because of the central role of dopaminergic neurotransmission, we used the individual local maxima of activation within the midbrain cluster (including VTA) as revealed by the contrast ABS > REL. To assess the coupling between midbrain and other brain areas, depending on the presentation of the alcohol-related stimuli, the CUE_{alc} block vs the $IAPS_{neutral}$ block were chosen as the psychological factors.

To this end, for each subject, the first eigenvariate time series from this position was extracted, adjusted for the effects of interest (corrected for variance explained by scrambled pictures and by head movements), and deconvolved with the canonical hemodynamic response function.[53] The PPI term was then defined as the element-by-element product of the neuronal time series and a vector coding of +1 for the CUE_{alc} block and –1 for the $IAPS_{neutral}$ block and of 0 for all other cases. Finally, the PPI term was reconvolved with the canonical hemodynamic response function.

New single-subject general linear models were set up that included the PPI term as the primary regressor of interest, as well as the eigenvariate time series from the midbrain, the psychological variable (ie, the weighted linear combination of CUE_{alc} and $IAPS_{neutral}$), the regressor modeling the scrambled picture presentation, the 6 rigid-body movement parameters, and a constant representing the mean over scans as covariates of no interest. The contrast images of the PPI term for all subjects was subjected to a second-level random-effects analysis.

COVARIATION FOR POTENTIAL CONFOUNDING VARIABLES

To account for differences in demographic and clinical variables, we included age, sex, number of cigarettes smoked per day, and Hamilton Depression Rating Scale score in all analyses. In VBM analysis, we also included the individual intracranial volume as calculated by VBM8. Furthermore, we addressed differences in global (SPM) or local gray matter volume (BPM). In SPM, the whole-brain gray matter volume divided by the intracranial volume as provided by VBM8 was used as a regressor, whereas, in BPM, tissue probability maps, as also provided by VBM, were used.

STATISTICAL THRESHOLDS

Based on the literature,[1,11-19] our analyses focused on key brain structures associated with cue-induced brain activity in alcohol dependence: a priori regions of interest for small-volume α error adjustments were created by combining anatomical hypotheses with functional findings as reported in the literature of comparable experimental designs for the MPFC, the ACC, the OFC, the amygdala, the VTA, and the ventral striatum (**Figure 2**; eAppendix; eFigures 1 and 2). To test for group

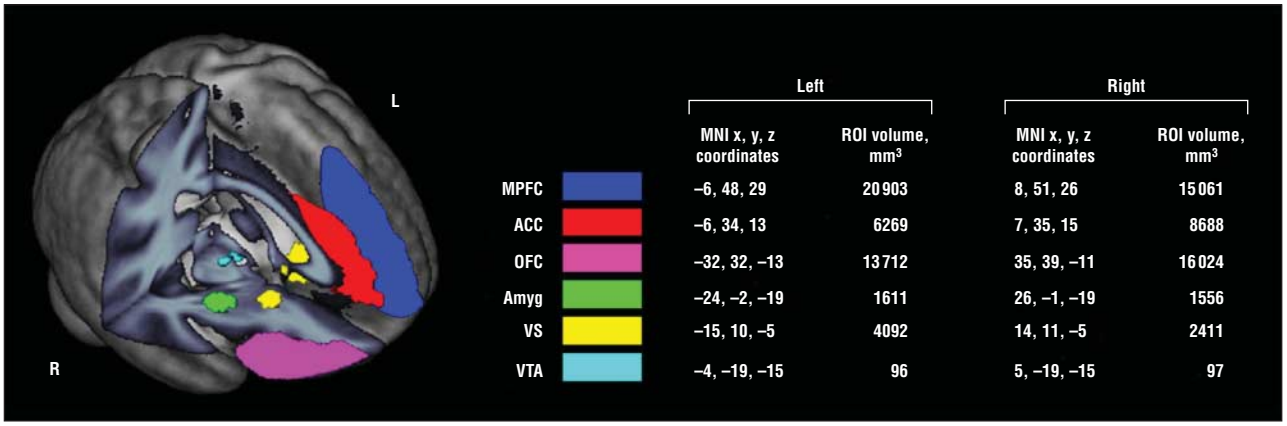

Figure 2. A priori–defined, literature-based probabilistic regions of interest (ROIs). The color-coded ROIs are overlaid on the averaged brain of the whole sample (neurological convention). A list of ROI Montreal Neurological Institute (MNI) x, y, z coordinates and ROI volumes are shown (eFigure 1). ACC indicates anterior cingulate cortex; Amyg, amygdala; L, left, MPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; R, right; VS, ventral striatum; and VTA, ventral tegmental area.

differences in these a priori–defined regions, a small-volume correction with a significance level set at $P<.05$ (familywise error–corrected) was used for all analyses. In further exploratory analyses, activations outside these predefined regions are reported at $P<.005$, with a cluster extent of at least 200 mm^3 (5 voxels for the fMRI analyses and 200 voxels for the VBM analysis), which are reported in eTables 1 to 10.

RESULTS

SAMPLE CHARACTERIZATION

Compared with healthy controls, alcohol-dependent patients reported stronger alcohol craving (on the Alcohol Craving Questionnaire: $t=-4.15$, $P<.001$), had a severity of alcohol dependence (on the Alcohol Dependence Scale: $t=-16.89$, $P<.001$), and had a higher severity of depression (on the Hamilton Depression Rating Scale: $t=-2.73$, $P=.01$). Moreover, the group of alcohol-dependent patients reported a significantly greater amount of lifetime alcohol intake (on the Lifetime Drinking History interview: $t=-7.13$, $P<.001$) and differed from healthy control group in socioeconomic status (Hollingshead Index of Social Position: $t=2.41$; $P=.02$). The groups also differed in smoking behavior (cigarettes smoked per day: $t=-6.55$, $P<.001$) (Table 1). The groups of prospective relapsers and abstainers did not differ in any of the listed measurements ($P>.15$ for all), except for the Hollingshead Index of Social Position, for which abstainers showed a lower socioeconomic status than relapsers ($t=2.22$; $P<.03$) (Table 2).

IMAGING DATA FROM VBM

The analysis of covariance revealed a significant main effect for the factor "group," with broad effects in cortical and subcortical regions. Compared with the healthy control group, the group of prospective relapsers revealed a pronounced atrophy in cortical midline structures, including almost all the regions of interest (the bilateral MPFC, the bilateral ACC, the bilateral OFC, the left amygdala, the bilateral ventral striatum, and the left VTA) (**Table 3**; **Figure 3**). Compared with the healthy control group, the group of abstainers showed a similar pattern but showed lower levels of cortical and subcortical atrophy when compared with the group of relapsers (Table 3; Figure 3). Compared with the group of abstainers, the group of relapsers revealed significantly less gray matter volume in the bilateral OFC, the right ACC, and the right MPFC (Table 3; Figure 3).

IMAGING DATA FROM BPM: NEURAL ACTIVITY DURING PROCESSING OF ALCOHOL-RELATED STIMULI (CORRECTED FOR LOCAL BRAIN ATROPHY)

Relapsers vs Controls: Alcohol-Associated Stimuli vs Control Condition

The second-level random-effects analyses conducted with BPM for the contrast image $CUE_{alc}>IAPS_{neutral}$ revealed an increased BOLD signal in the left MPFC in relapsers vs controls (peak coordinate in MNI space: –1, 32, 48); $z=4.49$, $P=.011$ [whole-brain familywise error–corrected]) (Table 3; **Figure 4**). For the comparison between controls and relapsers, no effects toward alcohol vs neutral cues could be found. For the comparison between controls and abstainers and between abstainers and controls, no effects toward alcohol cues could be found within our regions of interest.

Abstainers vs Relapsers: Alcohol-Associated Stimuli vs Control Condition

Compared with abstainers, relapsers showed an increased BOLD response during the processing of alcohol cues vs neutral pictures in the left MPFC (peak coordinate in MNI space: –1, 32, 48; $z=3.10$, $P=.008$) (Table 3; Figure 4). On the other hand, abstainers showed an increased brain response in the right VTA (peak coordinate in MNI space: 6, –18, –12; $z=3.17$, $P=.003$), in the left ventral striatum (peak coordinate in MNI space: –5, 11, –5; $z=3.44$, $P=.006$), and in the right ventral striatum (peak coordinate in MNI space: 10, 18, 3; $z=3.72$, $P=.005$) (Table 3; Figure 4).

Table 3. Summary of ROI Small-Volume FWE $P<.05$ Correctable Results[a]

	Gray Matter Volume			Cue Reactivity			Functional Connectivity	
ROI	CON vs REL	CON vs ABS	ABS vs REL	REL vs CON	REL vs ABS	ABS vs REL	ABS vs CON	ABS vs REL
MPFC								
R	<.001[b]	.010[c]	.02[c]					
L	<.001[b]	.010[c]		.011[b]	.008[c]			
ACC								
R	<.001[b]	.001[c]	.04[c]					
L	<.001[b]	.002[c]						
OFC								
R	.001[c]	.004[c]	.002[c]					
L	<.001[b]	.008[b]	.008[c]				.005[c]	.008[c]
Amygdala								
R								
L	.010[c]	.008[c]						.011[c]
VS								
R	<.001[c]	.010[c]				.005[c]		
L	.005[c]	.036[c]				.006[c]		
VTA								
R						.003[c]		
L								

Abbreviations: ABS, abstainers; ACC, anterior cingulate cortex; CON, controls; FWE, familywise error; L, left; MPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; R, right; REL, relapsers; ROI, region of interest; VS, ventral striatum; VTA, ventral tegmental area.

[a] Brain response $CUE_{alc} > IAPS_{neutral}$ as revealed by Biological Parametric Mapping analysis.

[b] Familywise error–corrected for the whole brain.

[c] Familywise error–corrected for the small volume.

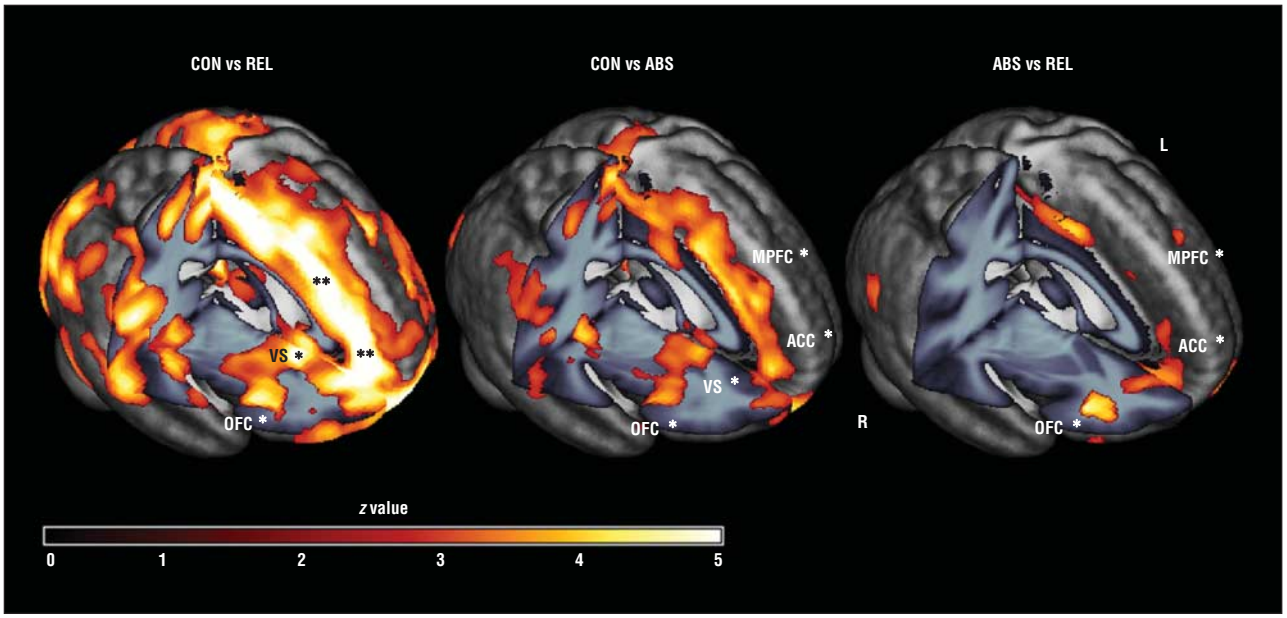

Figure 3. Differences in local gray matter volume computed by voxel-based morphometry. Group differences are overlaid on the averaged brain of the whole sample. ABS indicates abstainers; ACC, anterior cingulated cortex; CON, controls; L, left; MPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; R, right; REL, relapsers; and VS, ventral striatum. *Familywise error–corrected $P<.05$ for comparison between groups, adjusted for literature-based probabilistic regions of interest. **Familywise error–corrected $P<.05$ for comparison between groups, adjusted for the whole brain (Figure 2).

IMAGING DATA FROM PPI ANALYSES

A modulatory effect of alcohol-associated cues ($CUE_{alc}>IAPS_{neutral}$) on the functional connectivity between the right midbrain (the seed region) and other brain region was found in the comparisons between abstainers and relapsers and in the comparisons between abstainers and controls. Compared with healthy controls, abstainers revealed an increase in functional connectivity between the right midbrain and the left OFC (peak coordinate in MNI space: –40, 32, –1; $z=3.87$, $P<.001$). Compared with the group of relapsers, the group of abstainers showed an increase in functional connectivity between the right midbrain and the left OFC for alcohol-associated cues (peak coordinate in MNI space: –43, 21, –12; $z=3.55$, $P<.001$) and between the right midbrain and the left amygdala for alcohol-associated cues (peak coordinate in MNI space: –33, –4, –15; $z=3.48$, $P=.011$) (**Figure 5**).

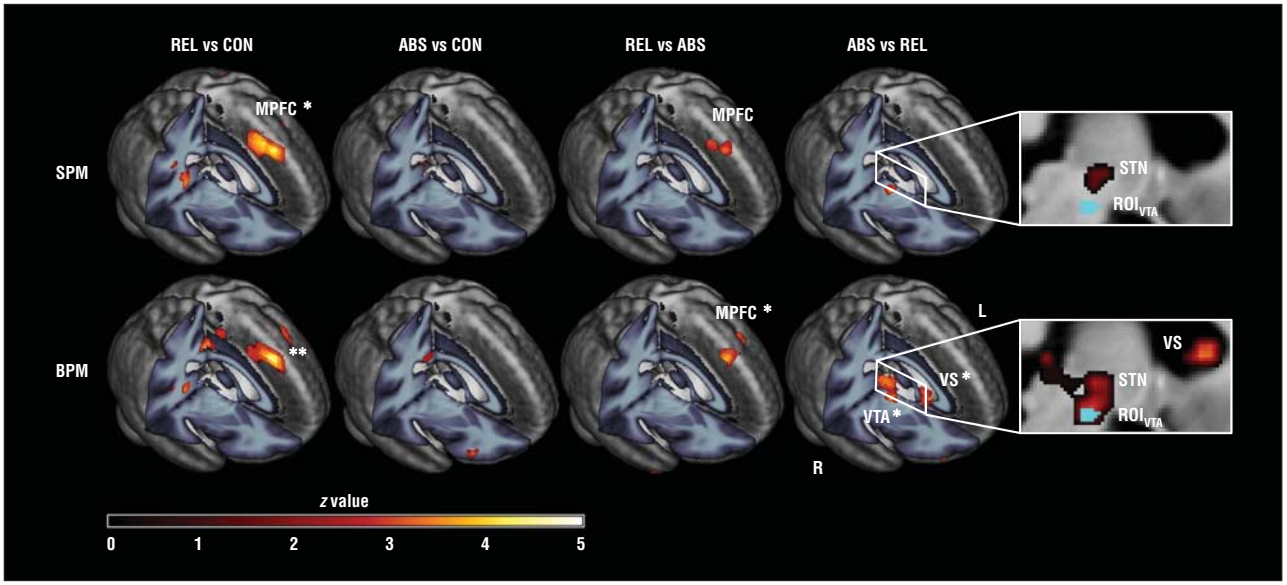

Figure 4. Neural responses to alcohol-related cues as measured by blood oxygen level–dependent function magnetic resonance imaging. The Statistical Parametric Mapping (SPM) and Biological Parametric Mapping (BPM) imaging data were corrected for global and local gray matter volume, respectively. ABS indicates abstainers; CON, controls; L, left; MPFC, medial prefrontal cortex; R, right; REL, relapsers; STN, subthalamic nucleus; VS, ventral striatum; and VTA, ventral tegmental area. *Familywise error–corrected $P<.05$ for comparison between groups, adjusted for literature-based probabilistic regions of interest. **Familywise error–corrected $P<.05$ for comparison between groups, adjusted for the whole brain (Figure 2).

COMMENT

The present findings suggest that pronounced atrophy in cortical midline structures and an increased brain response in the MPFC during processing of alcohol-related stimuli are associated with the prospective relapse in detoxified alcohol-dependent patients. On the other hand, an increased cue-induced brain response in the midbrain (including the VTA and the subthalamic nucleus [STN]) and an enhanced functional connectivity between the midbrain and the amygdala and between the midbrain and the OFC appear to be protective against relapse.

Among prospective relapsers, morphometric analyses revealed pronounced tissue loss in cortical midline structures and in subcortical regions. Abstainers showed similar but less severe patterns of cortical and subcortical volume reductions. These results are in line with several studies[54-58] showing distinctive gray matter loss in alcohol-dependent patients. Rando et al[25] observed reduced gray matter volume in the medial frontal and posterior parietal-occipital brain regions in detoxified patients, and this reduction was predictive for early relapse. Moreover, volume reductions in several brain regions, including the superior frontal and cingulate gyri and the amygdala, were found in alcohol-naive high-risk subjects, which suggests that at least part of the volume reductions could be preexisting and predispose to excessive alcohol intake rather than just resulting from neurotoxic effects.[26] In our data, there is also a significant reduction of gray matter volume in relapsers compared with abstainers in the MPFC, the OFC, and the ACC. On a functional level, the OFC, the ACC, and the MPFC have been shown to be involved in inhibitory control, decision making, reward prediction, salience attribution, and hedonic experience.[59,60] Regarding the OFC, studies[61,62] in nonhuman primates showed that lesions on the OFC result in impairments of reversal learning of stimulus-reinforcement associations, which can lead to perseveration and resistance to extinction of reward-related behaviors. In human studies,[63,64] lesions in the orbitofrontal cortical regions were closely linked to impulsivity, risk-taking behavior, and impaired goal-directed behavior. Our findings could thus help to explain why alcohol-dependent patients with OFC volume reduction may misjudge risky situations or spontaneously consume alcohol despite the conscious decision not to do so. Indeed, several studies[65,66] have consistently shown a reduction in frontal metabolism, including the OFC, in alcohol-dependent subjects compared with healthy controls. Interestingly, Tanabe et al[67] observed lower gray matter volume in the bilateral medial OFC of alcohol-dependent subjects, which was correlated with pathological decision making (ie, high-risk behavior in a gambling task), although they had been long-term abstainers. Dysfunctional ACC activation by alcohol-associated cues may bias attention toward alcohol-related stimuli and impair error monitoring and extinction of previously rewarding behavior (ie, alcohol consumption).[68,69] Taken together, brain volume deviations in the OFC, the ACC, and the MPFC may impair extinction processes of previously rewarding alcohol consumption and inhibition of drug seeking and intake.

The functional BPM analyses (which takes individual differences in brain volumes into account in a voxel-by-voxel manner) revealed an increased BOLD response during the processing of alcohol cues in the left MPFC in relapsers compared with healthy controls and in relapsers compared with abstainers. This finding is in line with previous research[1,21,70] showing an enhanced cue-induced brain response in this area, which may mediate an attentional bias toward drug cues. Interestingly, the

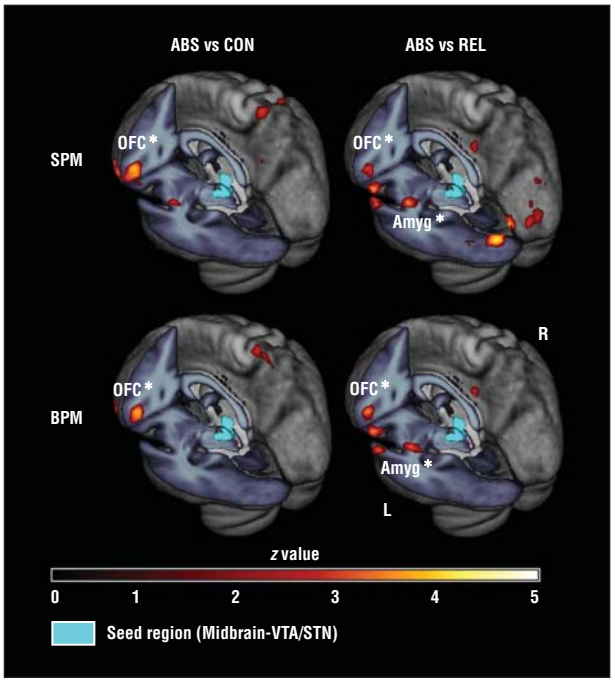

Figure 5. Brain regions showing an increase in functional connectivity for alcohol-associated cues with the midbrain (including the ventral tegmental area [VTA] and the subthalamic nucleus [STN]) (the seed region, which is shown in cyan). The Statistical Parametric Mapping (SPM) and Biological Parametric Mapping (BPM) imaging data were corrected for global and local gray matter volume, respectively. ABS indicates abstainers; CON, controls; L, left; OFC, orbitofrontal cortex; R, right; and REL, relapsers. *Familywise error–corrected $P < .05$ for comparison between groups, adjusted for literature-based probabilistic regions of interest.

observed brain response here is more superior than the previous finding from our group[1]; this might be an effect of sample size or of differences in the covariables used for correction (local gray matter volume, as well as age, sex, smoking behavior, and mood effects). Notably, the MPFC is rather large and putatively functionally heterogeneous. Therefore, further studies should explore the specific roles of functional subdivisions within this brain area that are related to cue processing and the risk of relapse. Using fMRI in nonaddicted but heavy drinkers, Kareken et al[71] showed that a family history of alcoholism significantly affects medial frontal responses to the odors of a subject's preferred alcoholic drinks. Subjects with a positive family history for alcoholism displayed a greater response to alcoholic drink odors than did subjects with a negative family history. Moreover, subjects with a genetic disposition toward alcohol dependence (*GABRA2* high-risk allele carriers) showed an increased BOLD response in the MPFC when inhaling alcohol-associated odors and a weaker alcohol-odor response in the VTA.[13]

Compared with the group of relapsers, the group of abstainers showed an increased brain response in the right midbrain. The midbrain region implied in our findings includes the VTA and the STN, brain areas closely linked with dopaminergic regulation of the ventral striatum, a core area of the brain reward system.[72]

Evidence for involvement of the STN in the regulation of behavior in conflicting choices comes from human[73] and animal[74] studies. Fleming et al[73] found increased STN activity and strengthened modulation of this activity by the OFC, when default behavior was inhibited in the face of heightened decision difficulty. In the context of addiction, the default behavior usually is associated with drug seeking and intake. In turn, abstinence equals the rejection of the default behavior in alcohol-addicted patients. In this light, STN activity in our abstainers might reflect this ability for a successful inhibition. Moreover, STN activity seems to also depend on addiction history. A study by Lardeux and Baunez[74] observed increased drug-seeking behavior in "high-drinker" rats after an STN lesion but increased water-seeking behavior in "low-drinker" rats after an STN lesion. The latter is in line with a missing STN activation on alcohol cues in our controls.

Ventral striatal activation was unexpected and only found when correcting for local gray matter volume. This may be the reason why other studies did not report such a finding. Zink et al[75] and Niznikiewics and Delgado[76] showed that the ventral striatum responds not only to appetitive signals, such as positive reinforcers, but also to negative reinforcers or saliency per se. Therefore, increased ventral striatal activation in prospective abstainers, in conjunction with intact or even increased connectivity between the reported midbrain structures and both the OFC and the amygdala, may indicate an intact value assessment of alcohol cues, including an appreciation of the positive and negative consequences of alcohol.

To the best of our knowledge, this is the first study powered to directly compare relapsers and abstainers. Other studies implicating the ventral striatum[16] or the central striatum[1] in alcohol-cue reactivity assessed correlations with alcohol intake rather than group differences[1] and assessed correlations with medication effects, not relapse rates.[16] In accordance with the hypothesis that functional activation of the VTA and the ventral striatum may also attribute saliency to aversive cues, Brischoux et al[77] observed in rodents that aversive stimuli (footshocks) led to a phasic excitation of dopaminergic neurons in the VTA. Moreover, beyond dopamine, local inhibitory responses in the VTA may produce significant BOLD responses.[78] In addition, midbrain-originated dopaminergic innervations of the amygdala were shown to be associated with increased limbic processing of aversive stimuli.[79] In line with this, our PPI analysis revealed that subsequent abstainers showed an increased connectivity between the midbrain (including the VTA and the STN) and the left amygdala and between the midbrain and the OFC for alcohol-associated stimuli (ie, regions known to be connected within the mesocorticolimbic dopamine system).[80] The amygdala is involved in the attachment of emotional valence to events (ie, reward and punishment)[81] and is thus crucial for decision making and learning.[82] The OFC is involved in inhibitory control, reward prediction, and salience attribution,[59,60] and, when impaired, subjects tend to perseverate and resist extinction of reward-related behaviors.[61,62] Individuals with amygdala lesions, on the other hand, fail to develop classically conditioned fear responses. In the context of addiction, Möller et al[83] investigated rats with basolateral amygdala lesions and observed an increase in voluntary ethanol consumption and a decrease in experimental induced anxiety. They suggested that cue-

induced activation of the amygdala may primarily be associated with negative affect and the unpleasant effects of previous alcohol intake and may not necessarily be associated with a cue-induced positive motivation to consume alcohol. Wrase et al[23] examined gray matter volume in a group of detoxified alcohol-dependent patients and revealed that a significant decrease of amygdala volume was associated with increased alcohol craving and an increased alcohol intake during a 6-month follow-up period. Makris et al[84] examined cocaine-dependent subjects who were also abusing alcohol and observed that amygdala volume reductions were associated with drug craving. This line of evidence indicates that amygdala function is crucial to evoke aversive affective states toward drug-related cues that may counteract craving and alcohol intake. In turn, preserved integrity of midbrain-amygdala-OFC connectivity could enable detoxified subjects to process aversive aspects of alcohol intake, which may help patients to abstain from further alcohol consumption. Therefore, the midbrain-amygdala connectivity may mediate aversive reactions to substance-related effects,[85] which may thus (when processed by the OFC) act as a "warning signal" that helps alcohol-dependent patients to abstain. In the light of this argument, it is interesting to note that the work by Kareken et al[13] raises the possibility that preexisting genetic mechanisms may alter the mesocorticolimbic circuit's sensitivity (including the VTA) to reward cues.

Possible limitations of the findings of our study are the differences in smoking behavior and negative mood states between the healthy controls and the alcohol-dependent patients, as well as differences between all subgroups in socioeconomic status. However, all statistical analyses remained significant when controlling for these variables. Furthermore, a genotype effect could not be assessed because of the limited sample size.[86,87]

In summary, we observed that preserved gray matter volume in the OFC, the MPFC, and the ACC, as well as cue-induced brain response in the midbrain comprising of the VTA and the STN, together with an increased functional connectivity between these 2 brain areas and the amygdala/OFC, appear to be associated with abstinence in detoxified alcohol-dependent patients, whereas increased cue-induced MPFC activation was related to an increased relapse risk. Preserved neural activation and connectivity between the VTA and the amygdala and between the VTA and the OFC, elicited by alcohol-associated stimuli, may help patients to sense the danger of situations in which alcohol is available. This functional activation may thus be perceived as a "warning signal," amplifying especially aversive aspects of alcohol cues, and may help patients to remain abstinent.

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Online-Only Material: The eAppendix, eTables, and eFigures are available at http://www.archgenpsychiatry.com.
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