Gene X Disease Interaction on Orbitofrontal Gray Matter in Cocaine Addiction

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Context: Long-term cocaine use has been associated with structural deficits in brain regions having dopamine-receptive neurons. However, the concomitant use of other drugs and common genetic variability in monoamine regulation present additional structural variability.

Objective: To examine variations in gray matter volume (GMV) as a function of lifetime drug use and the genotype of the monoamine oxidase A gene, MAOA, in men with cocaine use disorders (CUD) and healthy male controls.

Design: Cross-sectional comparison.

Setting: Clinical Research Center at Brookhaven National Laboratory.

 Patients: Forty individuals with CUD and 42 controls who underwent magnetic resonance imaging to assess GMV and were genotyped for the MAOA polymorphism (categorized as high- and low-repeat alleles).

Main Outcome Measures: The impact of cocaine addiction on GMV, tested by (1) comparing the CUD group with controls, (2) testing diagnosis X MAOA interac-

ions, and (3) correlating GMV with lifetime cocaine, alcohol, and cigarette smoking, and testing their unique contribution to GMV beyond other factors.

Results: (1) Individuals with CUD had reductions in GMV in the orbitofrontal, dorsolateral prefrontal, and temporal cortex and the hippocampus compared with controls. (2) The orbitofrontal cortex reductions were uniquely driven by CUD with low-MAOA genotype and by lifetime cocaine use. (3) The GMV in the dorsolateral prefrontal cortex and hippocampus was driven by lifetime alcohol use beyond the genotype and other pertinent variables.

Conclusions: Long-term cocaine users with the low-repeat MAOA allele have enhanced sensitivity to gray matter loss, specifically in the orbitofrontal cortex, indicating that this genotype may exacerbate the deleterious effects of cocaine in the brain. In addition, long-term alcohol use is a major contributor to gray matter loss in the dorsolateral prefrontal cortex and hippocampus, and is likely to further impair executive function and learning in cocaine addiction.

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ologic DA levels in the synapse, such as cocaine, might cause persistent cellular changes resulting in reduced neural volume compared with nonexposed individuals. Moreover, positron emission tomography studies have shown that the reduction in brain metabolism in DLPFC, OFC, and anterior cingulate cortex in cocaine abusers is associated with loss of postsynaptic DA markers.

Addiction to crack cocaine involves long-term concurrent use of other substances that are known to influence brain morphologic characteristics. More than 60% of individuals with CUD also had a comorbid alcohol use disorder and more than 80% smoked cigarettes, further compounding GM loss throughout the brain. These high comorbidity rates make the assessment of long-term drug use other than cocaine imperative for the generalizability of the results to community samples of individuals with CUD. Therefore, the present study used MR imaging and whole-brain voxel-based morphometry (VBM) analysis to test changes in cerebral GMV as a function of CUD and in correlation with the chronicity of lifetime drug use. This analysis, however, does not indicate whether the predicted structural alterations result uniquely from years of chronic drug use. It is possible that individuals with CUD had reduced DA and reduced neural volume in the relevant brain circuits before disease onset, which could have predisposed them to drug use and addiction. The potential contribution of genetic differences to GMV may be present before disease onset and may interact with long-term drug use, rendering some individuals with CUD more sensitive to GM loss than others.

Genetic variations that interact with and affect brain development may contribute to behaviors that increase addiction liability. The product of the monoamine oxidase A gene, MAOA, is an enzyme that regulates the metabolism of monoamine neurotransmitters, thereby modulating brain function and structure. During prenatal development, the MAOA enzyme is crucial for catabolic degradation of DA and norepinephrine, inducing changes with long-term consequences during childhood. The MAOA genotype (defined as OMIM +309850), a variable number tandem repeat (uVNTR) region, is divergent in primates, suggesting that it plays a pivotal role in differential MAOA expression in both humans and monkeys. The MAOA genotype is relevant to GMV in healthy CON. In a large VBM study, healthy carriers of the low-repeat allele of MAOA (MAOA*H) had reduced GMV in the cingulate cortex and bilateral amygdala and increased GMV in the OFC compared with high-repeat allele (MAOA*L) carriers. Furthermore, in the presence of extreme environmental challenge (childhood abuse), MAOA*L genotype increases the risk of antisocial behaviors in adulthood, pointing to a gene × environment interaction. Studies have also suggested association of MAOA*L with the risk of alcohol addiction. We reasoned that, for individuals with CUD, the disease onset and its progression could be viewed as an environmental challenge, possibly influencing GMV in affected members of the MAOA*L genotype (CUD-L group).

Therefore, in this study we predicted a main effect of addiction by which individuals with CUD would have reductions in GMV compared with CON. Next, we hypothesized a gene × disease interaction driven mostly by GMV loss in the CUD-L group. We hypothesized that a model containing both genetic and long-term drug use variables would better explain the predicted morphologic deficits in CUD.

METHODS

PARTICIPANTS

Eighty-two right-handed men (40 with CUD and 42 CON) were recruited by advertisement in local newspapers. All participants provided informed consent in accordance with the local institutional review board. Physical/neurologic, psychiatric, and neuropsychological examinations were conducted and included tests of intellectual functioning (Wide-Range Achievement Test 3 reading and the Matrix Reasoning subset of the Wechsler Abbreviated Scale of Intelligence), Beck Depression Inventory (BDI), to assess symptoms in the past 2 weeks, the Addiction Severity Index, and the Structured Clinical Interview for DSM-IV Axis I Disorders (research version). All participants were healthy, were not taking any medications, and were excluded if they had contraindications to the MR imaging environment (eg, metal in the body or claustrophobia), history of head trauma or loss of consciousness (>30 minutes), other neurologic disease, abnormal vital signs at time of screening, history of major medical conditions (cardiovascular, endocrinologic, oncologic, or autoimmune diseases), major psychiatric disorders (other than cocaine dependence and alcohol abuse for the CUD group and/or nicotine dependence for both groups), and urine positive (by means of a urinalysis kit [Biospsych; Biopsych Triage, San Diego, California] for psychoactive drugs or their metabolites [phenycyclidine, benzodiazepines, amphetamines, cannabis, opiates, barbiturates, and inhalants] except for cocaine in CUD.

All participants in the CUD group were current users: urine was positive for cocaine in all but 6 of the 40 individuals, and they reported use a mean (SD) of 2.1 (1.5) days before the study. Current use or dependence on other drugs was denied and corroborated by preimaging urine tests in all participants (urine was negative for all other drugs in all participants). Table 1 contains the demographic and clinical comparisons between the CUD and CON groups with nested genotype comparisons.

GENOTYPING

The DNA samples for MAOA genotyping were extracted from whole blood (PAXgene Blood DNA Kit; Qiagen Inc, Valencia, California) from each participant. The polymerase chain reactions were performed as previously described. In humans and primates, categorization of common genetic variability is based on a functional polymorphism in the promoter region of the MAOA gene; uVNTR, 3.5 or 4 repeats (ie, “high”) and 2, 3, or 5 repeats (“low”) is common in the population in whom 3 and 4 occur in a ratio of approximately 60:40 in men. Compared with the high variant, the low variant has relatively lower transcriptional activity in human nonneural cell lines. In this sample, alleles were observed in expected ranges by means of GeneScan version 3.7 and Genotyper version 3.6 software (both Applied Biosystems, Carlsbad, California). Genetic analyses resulted in 42 participants classified as having the low-MAOA-repeat alleles (22 CUD-L and 20 CON-L) and 40 as having the high-repeat alleles (18 CUD-H and 22 CON-H).

MR IMAGE ACQUISITION AND VOXEL-BASED MORPHOMETRY

All participants underwent T1-weighted anatomic MR imaging on a 4-T imager (Varian/Siemens, Malvern, Pennsylvania), with...
Table 1. Demographic and Drug Exposure Factors

<table>
<thead>
<tr>
<th>Low (n=22)</th>
<th>High (n=18)</th>
<th>Low (n=20)</th>
<th>High (n=22)</th>
<th>Tests(^b)</th>
</tr>
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<tbody>
<tr>
<td><strong>Participant Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
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<td>45 (1)</td>
<td>40 (1)</td>
<td>38 (1)</td>
</tr>
<tr>
<td>Race, No. black/white</td>
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<td>17/1</td>
<td>14/6</td>
<td>12/10</td>
</tr>
<tr>
<td>Education, y</td>
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<td>13 (0.4)</td>
<td>13 (0.3)</td>
<td>14 (0.5)</td>
</tr>
<tr>
<td>SES</td>
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<td>34 (3)</td>
<td>34 (3)</td>
</tr>
<tr>
<td>Verbal IQ(^c)</td>
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<td>92 (3)</td>
<td>101 (3)</td>
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<td>Nonverbal IQ(^d)</td>
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<td>9 (1.0)</td>
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<td>BDI symptoms score</td>
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<td>9 (2)</td>
<td>2 (1)</td>
<td>5 (1)</td>
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<tr>
<td><strong>Drug Exposure Factors</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Age at CUD onset, y</td>
<td>24 (1.3)</td>
<td>28 (2.0)</td>
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<td>NA</td>
</tr>
<tr>
<td>Cocaine intake, g/occasion</td>
<td>11.0 (1.0)</td>
<td>16.0 (0.4)</td>
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<td>NA</td>
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<td>Cocaine use, y</td>
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<td>19 (1.3)</td>
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<td>NA</td>
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<tr>
<td>Cigarette smokers, No. (%)</td>
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<td>13 (72)</td>
<td>5 (25)</td>
<td>4 (18)</td>
</tr>
<tr>
<td>Alcohol abuse, No. (%)</td>
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<td>9 (1)</td>
<td>6 (1)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Alcohol consumption, oz</td>
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<td>17 (1)</td>
<td>17 (2)</td>
<td>19 (1)</td>
</tr>
<tr>
<td>Years of smoking</td>
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<td>22 (2)</td>
<td>4 (5)</td>
<td>2 (2)</td>
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<td>Years of drinking(^e)</td>
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<td>13 (72)</td>
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<td>Alcohol consumption, oz</td>
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<td>59 (6)</td>
<td>34 (1)</td>
<td>30 (3)</td>
</tr>
<tr>
<td>Age at alcohol abuse onset, y</td>
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<td>16 (1)</td>
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<td>NA</td>
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<tr>
<td>Age at CUD onset, y</td>
<td>19 (2.0)</td>
<td>17 (3.9)</td>
<td>5 (1.0)</td>
<td>2 (0.5)</td>
</tr>
</tbody>
</table>

Abbreviations: BDI, Beck Depression Inventory; CON, controls; CUD, cocaine use disorders; CUD-H, CUD with high-repeat monomamine oxidase gene (MAOA) genotype; CUD-L, CUD with low-repeat MAOA genotype; NA, not applicable; SES, socioeconomic status.

\(^a\) Values are mean (SEM) unless otherwise noted. “Low” and “High” indicate low- and high-repeat MAOA genotype.

\(^b\) Results of general linear model with significant results labeled (eg, CUD).

\(^c\) From the Matrix Reasoning subset of the Wechsler Abbreviated Scale of Intelligence.\(^34\)

\(^d\) From the Wide-Range Achievement Test, third edition.\(^33\)

\(^e\) The number of lifetime years of drinking (note that CON had years of drinking, although alcohol abuse was ruled out).

Sonata gradient set. The MR imaging variables of the 3-dimensional modified driven-equilibrium Fourier transform\(^9,40\) sequences were as follows: echo time/repetition time, 7/15 milliseconds; 0.94 × 0.94 × 1.00 mm\(^3\) spatial resolution; axial orientation; 256 readout; and 192 × 96 phase-encoding steps, within a 16-minute imaging time. The modified driven-equilibrium Fourier transform sequence is particularly effective for white matter (WM)–GM tissue differentiation.\(^41\)

All structural data were analyzed with MATLAB 7.0 (MathWorks, Inc, Natick, Massachusetts; http://www.mathworks.com) and statistical parametric mapping (SPM5; Wellcome Department of Cognitive Neurology, London, England; http://www.fil.ion.ucl.ac.uk/spm) with VBM5.1 toolbox (Christian Gaser, PhD, Department of Psychiatry, University of Jena, Jena, Germany; http://dbm.neuro.uni-jena.de/vbm/). Preprocessing (spatial normalization, tissue segmentation, and bias correction) was conducted by means of a unified model. Images were normalized to standard proportional stereotactic space (Montreal Neurological Institute). Tissue probability maps (International Consortium for Brain Mapping, European version) were subsequently applied, segmenting the images of all 82 participants into GM, WM, and cerebrospinal fluid (CSF) tissue classes for each individual following Bayesian rule.\(^42,43\) A hidden Markov random field\(^44\) was applied to minimize the noise level by “removing” isolated voxels of one tissue class that are unlikely to be members of this tissue class, thus increasing the accuracy of the individual participant tissue probability maps. Finally, Jacobian modulation was applied to compensate for the expansion/contraction that occurs during nonlinear transformation and to restore the original absolute GMV in the segmented GM images. The voxel resolution after normalization was 1 × 1 × 1 mm. Statistical analysis of the regional GMV was performed after smoothing the normalized and modulated segments by means of an isotropic 12-mm\(^3\) full-width at half-maximum gaussian kernel.

Total brain tissue was computed as a sum of the extracted GMV and WM volume (WMV) for each participant. We did not analyze WMV in this study because other methods, such as diffusion tensor imaging, are more sensitive for this purpose (VBM’s WM T1 signal intensities are not correlated with the WM integrity).\(^45\) As in other studies,\(^46-48\) CSF was not used in the calculation for total brain tissue because the value outputs by SPM5 are susceptible to artifacts (eg, if voxels are not analyzed following Bayesian rule).\(^49\) In SPM5, general linear model 2 was used for the GM maps, controlling for total brain tissue and age, for the diagnosis main effect (CUD vs CON) and the genotype main effect (MAOA\(^L\) or >MAOA\(^H\)). Then, we conducted planned comparisons between CUD and CON of the same allele variation: CUD-L > CON-L and CUD-H > CON-H. Separate whole-brain regression analyses, controlling for total brain tissue and age, were

STATISTICAL ANALYSIS

Statistical analysis for the demographic and drug exposure factors was performed by means of a general linear model with a 2 (diagnosis: CUD vs CON) × 2 (genotype: low vs high) comparison or t tests or \(\chi^2\), as needed, in SPSS (SPSS, Inc, Chicago, Illinois).\(^50\) as documented in Table 1. In SPM5, general linear model 2 × 2 was used for the GM maps, controlling for total brain tissue and age, for the diagnosis main effect (CUD < CON) and the genotype main effect (MAOA\(^L\) or >MAOA\(^H\)). Then, we conducted planned comparisons between CUD and CON of the same allele variation: CUD-L < CON-L and CUD-H < CON-H. Separate whole-brain regression analyses, controlling for total brain tissue and age, were
then conducted to test associations between GMV and lifetime years of cocaine use (in the CUD sample [n=40], small-volume correction was used). Lifetime years of alcohol and cigarette use was evaluated in the whole sample (n=82). All SPM3 analyses were performed controlling for age and total brain tissue, with extent threshold of 100 voxels and a threshold set at P < .05, corrected with a false discovery rate equivalent to a T threshold of 3.3. Labels for the resulting coordinates were inspected by means of software (Anatomy Toolbox; Institute of Neuroscience and Medicine, Jülich, Germany) and a coplanar stereotactic atlas of the human brain.

The voxels of interest were extracted with SPM5 EasyROI toolbox (http://www.sbirc.ed.ac.uk/cyriu/cp_download.html) with an isotropic volume of the whole cluster around the significant peak voxel coordinates of the main effect results (CUD < CON from Table 2). This approach resulted in raw GMV values for each participant in each of these regions, allowing the measures to be used for figures and in SPSS to conduct general linear model analysis, covarying for total brain tissue, age, race, verbal intelligence, and BDI symptoms.

The model consisted of 2 hierarchical blocks: in the first block we entered total brain tissue, age, race, verbal intelligence, BDI, and MAOA. In the second block, we entered the lifetime drug use variables.

## RESULTS

### CHARACTERISTICS OF COCAINE ADDICTION

Individuals with CUD were significantly older than the CON group (mean [SEM] age, 45 [1] vs 39 [1] years), with no genotype effects (P = .28-.67). Additional differences included race (fewer whites in the CUD group than the CON group) and higher depression symptom score in CUD (9 [2]) than CON (3 [1]), with no genotype effects (P = .53-.89) and lower verbal intelligence (CUD, 90 [2]; CON, 97 [2]) and an interaction with the CUD-H group having lower scores than the CON-H group (P<.002). There were no differences between the groups in years of education and socioeconomic status (Table 1).

In terms of drug exposure factors, all participants with CUD used cocaine (smoked crack) in the past 0 to 7 days before imaging and met DSM-IV criteria for current cocaine dependence. The participants with CUD reported use of a mean (SEM) of 1.7 (0.8) g of cocaine per occasion with no genotype effects (P = .82). The years of lifetime cocaine use was 19 (1), with no genotype effects (P = .97). The age at CUD onset was 26 (1) years, and participants with CUD-L tended to be younger at onset (by approximately 4 years; P = .09, 2-tailed) than those with CUD-H. In addition to long-term cocaine use, the CUD sample also had a substantial lifetime use of cigarettes and alcohol. A larger proportion of individuals with CUD (30 [75%]) than CON (9 [21%]) reported cigarette smoking, with no difference in the number of cigarettes smoked per day (CUD, 8 [1]; CON, 5 [2]) and with no genotype effects (P = .32). In addition, 70% of the CUD group were also diagnosed as having alcohol abuse; their age at onset was 16 (1) years and they consumed 60 (8) ounces per occasion, with no genotype effects (P = .21).

### GM EFFECTS OF COCAINE ADDICTION

Total GMV was reduced with greater age across all participants (r = –0.30, P = .007) with no diagnosis or genotype effects (P = .85), and there were no main effects and no interactions in total WMV (P = .21). Controlling for age and total brain tissue, individuals with CUD had GMV reductions in the left OFC (Brodmann area [BA] 11) (F1,72 = 5.6; P = .002), right DLPFC (BA 9) (F1,72 = 27.5; P = .001), temporal cortex (BA 37) (F1,72 = 5.3; P = .02), and hippocampus and parahippocampus gyrus (F1,72 = 8.6; P = .002) compared with CON (CUD < CON; Table 2, Figure 1). The F values in parentheses throughout the “Results” section represent the main effects of addiction after controlling for the potential influences of total brain tissue, age, race, verbal intelligence, and BDI symptoms.

At this SPM threshold (P < .05, false discovery rate corrected), there were no regions of increased GMV in CUD compared with CON and no main effects of genotype as assessed with MAOA* L > or < MAOA* H contrasts. However, there was a significant CUD × MAOA interaction effect exclusively in the OFC (F1,68 = 3.2, P = .003).
GENE × DISEASE INTERACTION

After examining our planned contrasts and to investigate the source of the gene × disease interaction effect in the OFC, we matched the CUD and CON participants on allele variation (Table 2). Comparing CUD-H with CON-H (Figure 2, blue) demonstrated a diagnosis effect of GMV reductions in the hippocampus; however, this contrast did not produce significant results in any of the other main effect regions, including the OFC, even at a reduced threshold. Comparing CUD-L with CON-L (Figure 2, red) showed robust GMV reductions in the OFC, DLPFC, temporal cortex, and hippocampus, similar to the main effects of addiction. Here, however, the results not only included the OFC between the anterior branches of the medial and lateral orbital sulci (BA 11) but also encompassed the medial edge of the orbital surface, ie, gyrus rectus (Table 2). The general linear model SPSS analyses using our voxels of interest in these OFC coordinates in all participants, and controlling for the covariates as listed earlier, showed that the CUD-L group had significantly less GMV than the CUD-H group and both CON groups in the left OFC (MAOA × CUD; left, $F_{1,68}=10.6, P=.002$; right, $F_{1,68}=14.8, P=.001$) (Figure 2). This interaction was unique to the OFC (all other voxels of interest in Table 2, MAOA × CUD, $P=.10-.76$).

LIFETIME DRUG USE AND OTHER VARIABLES

To understand the contribution of drug use duration in this sample, we conducted multiple regressions in SPM5 of GMV with years of drug use, controlling for age and total brain volume. In the CUD group (n=40), with increasing years of cocaine exposure, there were more volume reductions in the OFC ($r=−0.44, P=.003$), DLPFC ($r=−0.41, P=.008$), and hippocampus ($r=−0.46, P=.003$); a similar pattern of results was obtained in the CUD group with lifetime alcohol (all $r=−0.34$ to $−0.65, P=.008-.001$) and with cigarette smoking (all $r=−0.31$ to $−0.52, P=.008-.001$) (Table 3, SPM results). In Figure 3, the whole-brain correlation results of all 3 drugs were overlaid, showing a visible overlap of the detrimental effects of all drugs on the hippocampus.

To understand the contribution of all the variables studied and the unique effects of long-term drug use, we conducted hierarchical regression analyses in SPSS. As
30% of unique variance. Notably, in the hippocampus striking, showing that lifetime alcohol use contributed groups. Results for the hippocampus were the most predictive of GM differences between the individuals. In the temporal cortex, race and alcohol use contributed the most unique variability to GMV, adding 24% to the 17% that was explained by the block 1 variables. In the DLPFC, lifetime alcohol and cocaine use significantly affected by any of the variables except for the known effect of reduced total GMV with greater age. As for the OFC, the block 1 variables contributed 21% to the GMV variance (driven by the MAOA genotype, age, and race). The drug use variables accounted significantly for an additional 19% of unique variance to the OFC GMV. This effect was driven by lifetime cocaine use. In the DLPFC, lifetime alcohol and cocaine use contributed the most unique variability to GMV, adding 24% to the 17% that was explained by the block 1 variables. In the temporal cortex, race and alcohol use were most predictive of GM differences between the groups. Results for the hippocampus were the most striking, showing that lifetime alcohol use contributed 30% of unique variance. Notably, in the hippocampus and DLPFC, alcohol and cocaine use contributed more variability than the block 1 variables.

**COMMENT**

These findings demonstrate a distributed pattern of GMV loss in participants with CUD compared with CON in the OFC, DLPFC, temporal, and hippocampal regions. Exclusively in the OFC, GMV reductions were driven by increasing years of cocaine use and by individuals in the CUD-L group having smaller GMV, showing a gene \times disease interaction. The pattern of GMV in other regions was not affected by the genotype; rather, GMV loss in the temporal region and especially the DLPFC and hippocampus was driven primarily by drug use, especially by alcohol use.

REDUCED GMV IN COCAINE ADDICTION

Participants with CUD had reduced GMV in the right dorsolateral region of the prefrontal cortex, in BA 9, a region critical for monitoring information in working memory and in the controlled retrieval of information. Specifically in CUD, these regions showed a deficit in functional activation during a go/no-go task, and deficits in these regions were associated with poor inhibitory control. With the use of measures of cortical thickness, this precise DLPFC region was found to be thinner in participants with CUD than well-matched CON participants. Additional GMV reductions were found in this study in the inferior posterior temporal cortex, BA 37, associated with object naming and recognition memory, and found to have reduced GMV in opiate-dependent individuals. This temporal region is particularly sensitive to age-dependent damage in Alzheimer disease. This region is located immediately adjacent to the posterior parahippocampal gyrus and the hippocampus, also found to have reduced GMV in those with CUD compared with CON in this study. The hippocampus plays a role in extinction of currently nonrelevant but previously rewarding stimuli and in retrieval of information.
pertinent to these learning mechanisms; as such, the hippocampus is implicated in drug-context memory and in relapse to drug-seeking behaviors. Together with the hippocampus, the regions found to have reduced GMV in CUD in the current study are associated with drug-craving and drug-seeking behaviors. Because the hippocampus, in concert with DLPFC regions, has an important executive role in inhibiting previously acquired drug reward mechanisms, these GMV decrements may perpetuate the impaired response inhibition and salience attribution syndrome in drug addiction.

The neurochemistry of these affected brain regions is modulated by tonic and phasic DA action. In humans, the in vivo concentration of DA receptors is related to neural volume, as demonstrated by a recent imaging study showing a voxel-wise relationship between DA D2 receptor availability (positron emission tomography with fallypride labeled with fluorine 18) and GMV in the DLPFC (BA 6 and 9) and temporal and parahippocampal gyrus, regions that were found to have reduced GMV in CUD in the present study. Medium spiny neurons are the principal targets of DA terminals, and DA depletion in animal studies results in neurons with shorter and fewer spines compared with nonexposed neurons. Because long-term drug use and addiction are associated with reduced DA D2 receptor availability, neuronal volume is predicted to be similarly reduced, as evident especially in prefrontal cortical DA projections from the ventral tegmental area. Studies in humans found a reduction of N-acetylaspartate (suggested as a putative marker for neuronal cell loss or damage) concentrations in CUD and increased levels of myoinositol (a marker of glial activation) in frontal cortical regions.

The present results demonstrate reduced volume of the OFC in the left hemisphere, whereas the rest of the main effect regions were right lateralized. These results may support the notion of a disrupted regional laterality in drug addiction, which is posited to be inherited.
it may start developing before disease onset and may indeed contribute to its onset and progression together with the influence of particular traits, such as impulsivity.73

GENE × DISEASE INTERACTION IN THE OFC

In this study, the CUD-L group had significantly smaller volume than the CUD-H group and both CON groups in the OFC and gyrus rectus (BA 11). The OFC has been implicated in a wide variety of externalizing behavior disorders, and patients with specific damage to the OFC demonstrate more impulsive behavior than patients with other prefrontal damage.74,75 The anterior part of the OFC consists of eulaminate (6-layer) cortex, including granular layer IV.76 Neurons in the OFC BA 11 of the macaque monkey code novelty, with rapid habituation,77 and BA 11 is strongly linked with DLPFC areas (also found in this study to have reduced GMV in CUD), which together may guide goal-directed motivation.78 The projections from the OFC to the entorhinal cortex, which innervates the pyramidal cells of the hippocampus, may underlie the process through which information about the emotional significance of stimuli is remembered.79

Limited GMV in the OFC may undermine its functional connections with dorsolateral and entorhinal regions, thereby impairing the ability to make advantageous decisions.80,81 Supporting poor connectivity is a study finding disruption in WM fiber tracts to the OFC in CUD, which may further impair the OFC connectivity to the DLPFC and hippocampus regions.82 The regional GM loss we documented herein may correspond to WM loss, which is more reliably documented in manual segmentation or diffusion tensor imaging studies than VBM.45

The selectivity of MAOA specifically on DA degradation may also be relevant during prenatal development, when MAOA is crucial for catabolic degradation of DA, norepinephrine, and perhaps also serotonin.83 Indeed, recent studies have shown that MAO (A and B) regulates neural progenitor cells during brain development, an effect mediated through serotonin.84 Dopamine depletion in adults, as reliably documented in CUD,3 can trigger large-scale gene expression changes through multiple regulatory subunit changes in messenger RNA expression levels.85 Although the MAOA uVNTR polymorphism analyzed in this study is not directly indicative of brain MAOA activity,86 this genetic variant was linked to the differences in levels of the DA metabolite homovanillic acid in CSF.87

It remains unknown whether the mechanisms by which decreased transcriptional activity of MAOA might increase GM in the OFC in healthy controls88 but interact with cocaine use to selectively diminish OFC in the present study. The modulating effect of the MAOA genotype on structural variability may have started during early brain development, clearly before disease onset, and possibly continued its effect at adolescence at onset of the disease process. Interestingly, the CUD-L group in this study had a slightly younger age at onset of cocaine use. It is possible that these individuals who later developed CUD had reduced GMV in the OFC before disease onset because developmental factors, such as maternal smoking, are associated with increased likelihood of drug experimentation and decreased thickness of the OFC in adolescence.89 In this context, it is noteworthy that the MAOA^L genotype was associated with risk of alcoholism and antisocial alcoholism.31 It is also noteworthy that other factors in addition to the MAOA polymorphism affect the enzyme’s expression. In a recent article, our group demonstrated that the MAOA gene is subjected to epigenetic modifications.90 This finding, together with the well-established evidence that the drugs of abuse cause epigenetic aberrations,91 led us to propose that the MAOA methylation pattern in CUD might be influenced by drug use, causing dysregulation of its expression.

Table 4. Contribution of Demographic, Genetic, and Drug Use Variables to GMVa

<table>
<thead>
<tr>
<th>Multiple Regression</th>
<th>Total GMV</th>
<th>OFC</th>
<th>DLPFC, BA 9</th>
<th>Temporal, BA 37</th>
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<tbody>
<tr>
<td>Block 1b</td>
<td>0.08 (.63), 44</td>
<td>0.21 (.62), 2</td>
<td>0.17 (.62), .02</td>
<td>0.24 (.62), .001</td>
<td>0.25 (.62), .008</td>
</tr>
<tr>
<td>Block 2c</td>
<td>0.02 (.59), .79</td>
<td>0.19 (.39), .004</td>
<td>0.24 (.59), .001</td>
<td>0.12 (.59), .05</td>
<td>0.30 (.59), .&lt;.001</td>
</tr>
<tr>
<td>Age</td>
<td>−0.20</td>
<td>0.206</td>
<td>0.02</td>
<td>0.05</td>
<td>−0.20</td>
</tr>
<tr>
<td>Race</td>
<td>0.07</td>
<td>0.25d</td>
<td>0.19</td>
<td>0.31e</td>
<td>0.14</td>
</tr>
<tr>
<td>Volume</td>
<td>NA</td>
<td>−0.08</td>
<td>0.07</td>
<td>0.07</td>
<td>−0.17</td>
</tr>
<tr>
<td>WRAT-3</td>
<td>0.08</td>
<td>−0.16</td>
<td>−0.11</td>
<td>0.00</td>
<td>−0.05</td>
</tr>
<tr>
<td>BDI</td>
<td>0.00</td>
<td>0.02</td>
<td>0.04</td>
<td>−0.02</td>
<td>−0.16</td>
</tr>
<tr>
<td>MAOA genotype</td>
<td>0.06</td>
<td>0.438</td>
<td>0.06</td>
<td>−0.02</td>
<td>−0.06</td>
</tr>
<tr>
<td>Lifetime cocaine use</td>
<td>−0.05</td>
<td>−0.408</td>
<td>−0.26d</td>
<td>−0.26</td>
<td>−0.20</td>
</tr>
<tr>
<td>Lifetime alcohol use</td>
<td>−0.06</td>
<td>−0.04</td>
<td>−0.558</td>
<td>−0.44e</td>
<td>−0.598</td>
</tr>
<tr>
<td>Lifetime smoking</td>
<td>−0.12</td>
<td>0.08</td>
<td>−0.13</td>
<td>−0.17</td>
<td>−0.20</td>
</tr>
</tbody>
</table>

Abbreviations: BA, Brodmann area; BDI, Beck Depression Inventory; DLPFC, dorsolateral prefrontal cortex; GMV, gray matter volume; MAOA, monoamine oxidase A gene; NA, not applicable; OFC, orbitofrontal cortex; WRAT-3, Wide-Range Achievement Test, third edition.33

a Unless otherwise indicated, values are standardized beta coefficients.
b Values are adjusted R^2, df, and P value (contains the variables age, race, total brain tissue, WRAT-3, BDI, and MAOA genotype).
c Values are adjusted R^2, Δ (change), df, and P value (contains the block 1 variables and block 2 variables plus years of cocaine use, alcohol abuse, and cigarette smoking with the corresponding standardized β coefficients).
d P < .05.
e P < .001.
Gray matter in the OFC, showing deficit in CUD-L, was uniquely driven by increasing years of cocaine exposure. In fact, the OFC was the only region affected specifically by cocaine and not years of alcohol use. It is possible that the OFC of individuals with CUD-L is more sensitive to the neurotoxic effects of cocaine than that of individuals with CUD-H exposed to similar amounts of the drug. Supporting this specificity is evidence from studies in rats showing that long-term stimulants limit spine density in the OFC (while long-term opiate use may increase spine density), perhaps making the OFC sensitive to morphologic changes depending on the drug of abuse. Additional morphometric damage can be caused by smoking exposure because long-term smoking inhibits MAO, and high-affinity nicotinic receptors in the human OFC increase after smoking. A recent VBM study showed that GMV in DLPFC and inferior frontal regions is reduced in cigarette smokers. However, consistent with the current results, nicotine administration to adolescent rats elicited less severe region-dependent effects than alcohol.

REGION-SPECIFIC EFFECTS OF LIFETIME ALCOHOL USE

Lifetime alcohol use was the major contributor of GM deficit in the DLPFC, temporal cortex, and hippocampus of participants with CUD, contributing unique variability to GMV above and beyond the MAOA polymorphism and any of the other factors tested, more so than cocaine and cigarette smoking. In this study, we measured severity as the number of lifetime years of use. Animal models of binge alcohol administration, controlling for severity in a dose-dependent manner, support a direct link between high levels of alcohol consumption and neurotoxic effects in the hippocampus and surrounding dentate gyrus and associated entorhinal-perirhinal cortex during adolescence. Similarly, reduced hippocampal volume was found among adolescents with alcohol use disorders. Gray matter loss in the hippocampus may lead to more drug seeking, as demonstrated by animal studies showing that blocking neurogenesis in the adult rat hippocampus caused increased cocaine seeking and more self-administration, further facilitating a vicious cycle of cocaine use. The observed GM reductions in the hippocampus, perhaps due to chronic alcohol use, may increase cocaine use through strong resistance to extinction of drug-seeking behavior.

CAVEATS

Our groups differed in age, ethnicity, verbal intelligence, and symptoms of depression. Demographic effects of difference in the lifetime trajectory of drug addiction are a source of variability and a contributor to the overall impact of the disease. Lower verbal intelligence could indicate compromised education due to drug use during adolescence (note that the differences due to genotype are partly supported by another study). The BDI measure (reflecting symptoms in the past 2 weeks) cannot be separated from drug effects (such as acute withdrawal). Rather than excluding these effects, we studied their impact in explaining GMV differences between the groups, enhancing the generalizability of the current results. On the subject of enhancing generalizability, it is important to remember that our findings come from a male sample; women are largely understudied in drug addiction, a limitation of generalizability that needs to be addressed in future studies. Our sample of individuals with CUD also underrepresented whites compared with African Americans, and the latter show GM effects in the OFC and temporal cortex. This represents a confounding factor in this study, but it also highlights the evidence of racial differences in GMV that need to be accounted for beyond the clinical variable of interest. Indeed, in this study we demonstrated through hierarchical regression analysis that the MAOA and cocaine use effects contribute unique variability to GMV beyond other effects.

Similarly, additional factors affect GM reductions, including, for example, long-term lack of sleep (affecting the OFC) and acute depression affecting hippocampal volume. Both are common problems in CUD and should be further investigated in future studies. The present study had active, currently using participants with CUD (90% had urine positive for cocaine), and a case could be made for OFC reductions during acute use that may recover with abstinence. However, even after prolonged abstinence of 2 to 4 years, GM reductions were still found in comparison of substance-dependent individuals and controls, pointing to persistent and enduring GM deficits in the OFC.

In a VBM study in healthy control participants, MAOA has had increased lateral OFC volume (BA 47) compared with MAOA. Conversely, in this study, the CUD-L group had significantly less GMV than CUD-H and both CON groups in the medial OFC and gyrus rectus. In the same previous study, healthy individuals with MAOA had reduced GM encompassing the entire cingulate gyrus and particularly in the anterior cingulate, a region not evident in the current results. While inspecting CON-L vs CON-H in our data, we could find a similar pattern including the anterior cingulate, using P < .05, uncorrected (results not shown). Differences in findings may stem from the use of varied methods with varied populations of controls and individuals with CUD. Other morphology studies found deficits in regions in which we did not find reduced GMV (eg, amygdala, anterior cingulate, and insula); conversely, none of the studies found the hippocampus GM deficits that we found in this sample, although we studied CUD with comorbid alcohol abuse, which has been associated with hippocampal volume loss. Future studies should continue to assess genotype differences within CUD because this study suggests CUD-L to be associated with potentially more extensive deficits than CUD-H (eg, earlier age at onset is a major risk factor for a more severe course of illness).

CONCLUSIONS

The extensive GMV loss in the OFC, DLPFC, temporal, and hippocampal regions in individuals with CUD underlies demographic, genetic, and drug use factors. Exclusively in the OFC, GMV reductions were driven by increasing years of cocaine use and by individuals with
CUD-L having smaller GMV, showing a gene × disease interaction. The population we studied had already started using drugs, which constrains the ability to track causes and effects of the substance abuse.8,21 Addiction liability can be characterized dimensionally among already affected individuals insofar as indexes of severity.22 These results suggest that loss of GMV among individuals with CUD is multidetermined and can be assessed with a model that includes genetic, behavioral, and drug use factors that we speculate have interacted continuously throughout the lifespan. Studies are emerging in support of this notion, that gene × environment interactions take different forms at different ontogenic stages of development during the lifespan.23,24 Therefore, the next generation of neurogenetic studies will have to document complex interactions over protracted developmental trajectories to explain the effects contributing to multifaceted psychopathology as drug addiction.

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