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ORIGINAL ARTICLE

Gray-matter volume, midbrain dopamine D2/D3 receptors and drug craving in methamphetamine users

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Dysfunction of the mesocorticolimbic system has a critical role in clinical features of addiction. Despite evidence suggesting that midbrain dopamine receptors influence amphetamine-induced dopamine release and that dopamine is involved in methamphetamine-induced neurotoxicity, associations between dopamine receptors and gray-matter volume have been unexplored in methamphetamine users. Here we used magnetic resonance imaging and [^{18}F]fallypride positron emission tomography, respectively, to measure gray-matter volume (in 58 methamphetamine users) and dopamine D2/D3 receptor availability (binding potential relative to nondisplaceable uptake of the radiotracer, BP_{ND}) (in 31 methamphetamine users and 37 control participants). Relationships between these measures and self-reported drug craving were examined. Although no difference in midbrain D2/D3 BP_{ND} was detected between methamphetamine and control groups, midbrain D2/D3 BP_{ND} was positively correlated with gray-matter volume in the striatum, prefrontal cortex, insula, hippocampus and temporal cortex in methamphetamine users, but not in control participants (group-by-midbrain D2/D3 BP_{ND} interaction, $P < 0.05$ corrected for multiple comparisons). Craving for methamphetamine was negatively associated with gray-matter volume in the insula, prefrontal cortex, amygdala, temporal cortex, occipital cortex, cerebellum and thalamus ($P < 0.05$ corrected for multiple comparisons). A relationship between midbrain D2/D3 BP_{ND} and methamphetamine craving was not detected. Lower midbrain D2/D3 BP_{ND} may increase vulnerability to deficits in gray-matter volume in mesocorticolimbic circuitry in methamphetamine users, possibly reflecting greater dopamine-induced toxicity. Identifying factors that influence prefrontal and limbic volume, such as midbrain BP_{ND}, may be important for understanding the basis of drug craving, a key factor in the maintenance of substance-use disorders.

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INTRODUCTION

A variety of studies have indicated that exposure to methamphetamine is associated with abnormalities in the mesocorticolimbic pathway. These include deficits in tyrosine hydoxylase,[1,2] dopamine transporter molecules[1,3] and dopamine D2/D3 receptor availability.[4–6] Furthermore, methamphetamine users who relapse during treatment have lower levels of dopamine D2/D3 receptor availability and dopamine release in the striatum than those who maintain abstinence,[7] suggesting that dopamine dysfunction may be clinically relevant. Chronic methamphetamine exposure is also associated with structural abnormalities in dopamine terminal fields, with lower gray-matter volume in the caudate nucleus, anterior cingulate, prefrontal cortex, insula, amygdala and hippocampus in methamphetamine users than healthy controls.[8–11] Despite evidence that methamphetamine-induced changes in dopaminergic markers are correlated with changes in gray-matter volume,[12] these associations have not been tested in humans. Understanding the mechanisms that underlie both neurochemical and structural deficits in mesocorticolimbic circuitry has the potential to help clarify addiction-related symptoms, such as drug craving, which persist in abstinent stimulant users.[13]

Methamphetamine-induced damage of dopaminergic neurons and neighboring cells has been linked to excessive levels of intracellular and intrasynaptic dopamine.[14–16] Methamphetamine increases cytosolic levels of dopamine, which is degraded to form oxidative metabolites that damage dopamine nerve terminals.[17] Mice lacking dopamine transporters (DAT knockouts) exhibit degeneration of GABAergic neurons in the striatum,[18] providing evidence that excessive extracellular dopamine can be neurotoxic. Furthermore, administration of D1- and D2-type receptor antagonists attenuates methamphetamine-induced loss of dopamine transporters and tyrosine hydroxylase as well as apoptosis in the striatum.[19] In humans, lower dopamine D2/D3 receptor availability in the midbrain, which has a high proportion of somatodendritic dopamine autoreceptors,[20] has been associated with greater amphetamine-induced striatum dopamine release.[21] This work suggests that regulation of dopamine release by somatodendritic dopamine autoreceptors in the substantia nigra and ventral tegmental area[22] may influence methamphetamine-induced neurotoxicity.

Dopamine signaling and brain function in regions that receive dopaminergic projections from midbrain have also been linked to drug craving. For example, cue-induced cocaine craving has been correlated with striatal dopamine release,[23,24] and drug-related cues elicit changes in brain activation in the orbitofrontal cortex, anterior cingulate,[25–29] ventral striatum,[25] amygdala[26,27,30,31] and insula.[25] Self-reported craving in these studies is positively correlated with activation in the striatum, orbitofrontal cortex, dorsolateral prefrontal cortex and anterior cingulate cortex.[32] A negative correlation between volume of the right inferior frontal

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gyrus and methamphetamine craving has been noted,[33] but associations of methamphetamine craving with gray-matter volume in other brain regions that receive dopaminergic innervation and/or with midbrain dopamine receptors have not been tested.

The aim of the current study was to examine the relationships between dopamine D2/D3 receptor availability (binding potential relative to nondisplaceable uptake of the radiotracer; BP_{ND}) using positron emission tomography (PET), gray-matter volume measured using high-resolution structural magnetic resonance imaging (MRI) and self-reported craving for methamphetamine. Previous studies have shown lower striatal dopamine D2/D3 BP_{ND} in methamphetamine users than in healthy controls;[4–6] a similar difference was expected regarding the midbrain. As indicated above, evidence suggests that excessive intrasynaptic dopamine is neurotoxic[14–16] and that midbrain D2/D3 BP_{ND} is negatively correlated with amphetamine-induced striatal dopamine release.[21] It was therefore anticipated that in methamphetamine users, but not in controls, lower midbrain D2/D3 BP_{ND} would be associated with smaller gray-matter volume in brain regions that receive dopaminergic projections from the midbrain. As methamphetamine-induced damage may similarly influence both dopamine receptors and morphometry in brain regions that receive dopaminergic innervation, a positive association between striatal D2/D3 BP_{ND} and striatal gray-matter volume in methamphetamine users was predicted. Furthermore, it was expected that lower midbrain D2/D3 BP_{ND} and lower gray-matter volumes in regions of the mesocorticolimbic system would be associated with greater methamphetamine craving.

METHODS AND MATERIALS

Participants

Ninety-five participants (58 methamphetamine users, 37 healthy controls) were included in this study. Participants were recruited through online and print advertisements, received a detailed explanation of the study (as approved by the UCLA Institutional Review Board) and gave written informed consent. Data from a subset of these participants were included in previous publications comparing methamphetamine and control participants on gray-matter volume[8] and striatal D2/D3 BP_{ND};[4] this study does not focus on previously reported group differences. A previous publication also used a subset of these participants to examine the correlation between methamphetamine craving and gray-matter volume in the right inferior frontal gyrus.[33] Here, we extend this analysis to the whole-brain assessments of gray-matter volume and midbrain D2/D3 BP_{ND}.

Exclusion criteria were as follows: central nervous system, cardiovascular, pulmonary or systemic disease; use of psychotropic medications, loss of consciousness >30 min, HIV seropositive status and pregnancy. The Structured Clinical Interview for DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th Edition)[34] was used to determine current axis I disorders. Control participants had no current axis I disorders except nicotine dependence. In addition to nicotine dependence, some methamphetamine users met the criteria for current marijuana dependence ($n=4$) or panic disorder ($n=1$). Methamphetamine users participated on a residential basis at the UCLA General Clinical Research Center (GCRC) and tested positive for methamphetamine in urinalysis at study entry. After admission, abstinence from methamphetamine, cocaine, opiates, marijuana and benzodiazepines was verified by urine testing. MRI and PET scanning were conducted during the first 4–7 days of abstinence from methamphetamine. Control subjects participated on an outpatient basis, and tested negative for methamphetamine, cocaine, opiates, marijuana and benzodiazepines on scan days.

Self-report of drug use and craving

Drug use and demographic variables were collected using a drug-use survey prepared for this study. Methamphetamine craving was assessed at intake by self-report. Specifically, participants were instructed to select the multiple of 10 between 0 ('not at all') and 100 ('strongest ever'), which best corresponded with their craving for methamphetamine in 'the past 24 h.' At intake, all methamphetamine users reported using methamphetamine within the last 5 days.

MRI acquisition

A magnetization-prepared rapid acquisition gradient echo sequence (TR = 1900 ms, TE = 4.38 ms, flip angle = 15°, FOV = 256 $\times$ 256 $\times$ 160, 160 slices, thickness: 1 mm) was run on a 1.5 T Siemens Sonata scanner (Siemens Medical Solutions, Erlangen, Germany) with a standard quadrature head coil to produce high-resolution, T1-weighted, MRIs.

MRI preprocessing

As outlined previously,[8] images were aligned to a standardized stereotactic space with the sagittal plane serving as the yz plane, the axial–oblique plane normal to this and containing the anterior and posterior commissures (AC–PC plane) as the xy plane and the coronal-oblique plane normal to the sagittal AC–PC planes serving as the xz plane. The origin of the space was set at the left-right and inferior-superior midpoint of the anterior commissure.

The voxel-based morphometry[35] toolbox (http://dbm.neuro.uni-jena.de/vbm/) for SPM8 (Wellcome Department of Imaging Neuroscience, London, UK) running on MATLAB 7.9 (Mathworks, Sherborn, MA, USA) was used to preprocess images. As described previously,[8] AC–PC aligned images are de-noised using an optimized blockwise non-local means de-noising filter.[36] Images were segmented into three classifications (gray matter, white matter and cerebrospinal fluid) using an adaptive maximum *a posteriori* technique[37] including partial-volume estimation.[38] A hidden Markov random field approach[39] was used to de-noise the data. Each image was registered to a standard template in Montreal Neurological Institute (MNI) space using Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra.[40]

The images were modulated by a procedure in which the intensity value of each voxel was multiplied by the local value of the Jacobian determinant of the deformation used to register each brain to MNI space. Linear components of the deformations, reflecting scaling due to head size, were not considered during modulation so that differences in volume because of head size would not affect intensity values. Intensity at each voxel ('gray-matter volume') reflected both the probability that the voxel contained gray matter and the local deformations required to register the brain to MNI space. Finally images were smoothed using an 8-mm full-width half maximum (FWHM) Gaussian kernel.

PET acquisition

Dopamine D2/D3 receptor availability was assayed using [^{18}F]fallypride[41] and a Siemens ECAT EXACT HR+ scanner (Siemens, Knoxville, TN, USA) (in-plane resolution FWHM = 4.6 mm, axial FWHM = 3.5 mm, axial field of view = 15.52 cm) in three-dimensional mode. A 7-min transmission scan was acquired using a rotating ^{68}Ge/^{68}Ga rod source for attenuation correction. PET dynamic data acquisition was initiated with a bolus injection of [^{18}F]fallypride (~5 mCi in 30 s). Emission data were acquired in two 80-min sessions, separated by a break to minimize participant discomfort and allow the participant to urinate to reduce radiation exposure to the bladder wall. Data were reconstructed using ECAT v.7.3 OSEM (ordered subset expectation maximization; 3 iterations, 16 subsets) after corrections for decay, attenuation and scatter.

PET preprocessing

Reconstructed PET data were combined into 16 sequential images, each containing an average of 10 min of data. PET images were corrected for head motion using rigid-body transformation with FSL FLIRT. Coregistration of the second PET image to the structural MRI using the ART software package was computed using a six-parameter rigid-body transformation and subsequently applied to all 16 PET images in the series.[42]

A midbrain volume of interest (VOI), encompassing the ventral tegmental area and substantia nigra, was delineated using previously described methods.[43] Bilateral caudate, putamen and nucleus accumbens VOIs were created using FSL FIRST (http://www.fmrib.ox.ac.uk/fsl/first/index.html).[44] Time–radioactivity data from VOIs were extracted from the motion-corrected, coregistered images and imported into PMOD 3.2 for kinetic modeling (PMOD Technologies, Zurich, Switzerland). Time–radioactivity curves were fit using SRTM (Simplified Reference-Tissue Model; Lammertsma and Hume[45]) to provide an estimate of $k2'$, the rate parameter for transfer of the tracer from the reference region to the plasma. As the cerebellum is nearly devoid of measurable specific binding sites for the radiotracer, a cerebellar VOI was used as a reference region.[46] A volume-weighted average of $k2'$ estimates from high-radioactivity regions (i.e., the caudate and putamen) was computed. The time–radioactivity curves were refit using the SRTM2[47] with the computed $k2'$ value held constant across all VOIs. BP_{ND} was then calculated by subtracting 1.0 from the product of the tracer delivery (R1) and the tracer washout ($k2'/k2a$).

Statistical analyses

Demographic and drug-use data were analyzed in SPSS 21 (IBM Corp, Armonk, NY, USA). As appropriate, Student's *t*-tests and χ^2 tests were used to evaluate group differences in these variables. Bivariate correlations and Student's *t*-tests were used to examine the relationships between methamphetamine craving and the demographic variables (Table 1). Univariate analysis of covariance, with midbrain D2/D3 BP_{ND} as the dependent variable, group as a between-subjects factor and age, sex, smoking status and frequency of marijuana use as covariates, was used to examine group differences in midbrain D2/D3 BP_{ND}. Partial correlations between methamphetamine craving and midbrain or striatal D2/D3 BP_{ND} included sex, days since last methamphetamine use and frequency of methamphetamine use as covariates.

To test the group-by-midbrain D2/D3 BP_{ND} interaction on gray-matter volume, FSL's Randomise (http://www.fmrib.ox.ac.uk/fsl/randomise, version 5.1) was used to implement voxelwise permutation-based nonparametric inference for the analysis of covariance, with age, sex, smoking status and frequency of marijuana use as covariates. A second model with the same covariates was used to test the group-by-striatal D2/D3 BP_{ND} interaction on gray-matter volume. The relationship between methamphetamine craving and gray-matter volume was examined using nonparametric inference for linear regression including sex, days since last methamphetamine use and frequency of methamphetamine use as covariates. For each of the contrasts tested, 10 000 permutations were performed. Threshold-free cluster enhancement was used to correct for multiple comparisons, and a statistical threshold of $P < 0.05$ was applied to each of the resulting statistical maps.

RESULTS

Participant characteristics

The subset of participants in the methamphetamine ($n = 31$) and control groups ($n = 37$) with assessments of dopamine D2/D3 BP_{ND} did not differ in age ($t(66) = 1.69$, $P = 0.10$), sex (χ^2 (68) = 1.87, $P = 0.17$), days of alcohol use in the 30 days preceding study entry ($t(66) = 0.088$, $P = 0.93$), number of alcoholic drinks per week ($t(66) = 0.41$, $P = 0.68$) or pack-year smoking history ($t(66) = -1.33$, $P = 0.19$). The methamphetamine group smoked marijuana more frequently ($t(66) = -3.0$, $P = 0.004$) and included more tobacco cigarette smokers than the control group (χ^2 (68) = 5.81, $P = 0.02$). On average, participants in the methamphetamine group ($n = 58$) used methamphetamine on 22.1 ± 7.8 days in the 30 days preceding study entry and had used methamphetamine heavily for 7.9 ± 6.8 years (heavy use defined as three times a week or at least one 2-day binge per week) (Table 1).

Relationships between gray-matter volume and midbrain or striatal D2/D3 BP_{ND}

Methamphetamine and control groups did not differ in midbrain D2/D3 BP_{ND} ($F(1,62) = 0.76$, $P = 0.39$, Figure 1; control group mean: 1.11, s.d.: 0.34; methamphetamine group mean: 1.13, s.d.: 0.26). Whole-brain, voxelwise analysis revealed significant group-by-midbrain D2/D3 BP_{ND} interactions on gray-matter volume of bilateral striatum, insula, temporal cortex, left dorsolateral prefrontal cortex, hippocampus, orbitofrontal cortex and right

Table 1. Participant characteristics

Demographic and drug-use characersitics[a]	*Methamphetamine-dependent group (n = 58)*	*Subset with [^{18}F]fallypride BP_{ND}*	
		Healthy control group (n = 37)	*Methamphetamine-dependent group (n = 31)*
Age (years)	32.8 ± 9.2	36.8 ± 1.4	33.2 ± 1.7
Sex (no. of male/female)	28/30	24/13	15/16
Frequency of alcohol use[b]	6.1 ± 8.6	4.4 ± 1.1	4.2 ± 1.2
Average no. of alcoholic drinks per week	1.8 ± 0.3	1.7 ± 0.4	1.5 ± 0.3
Frequency of marijuana use[a,c]	4.2 ± 8.4	0.3 ± 0.3	4.0 ± 1.4
Number of cigarette smokers[d]	54	21	26
Pack year smoking history	9.9 ± 12.9	6.6 ± 1.7	10.6 ± 2.5
Frequency of methamphetamine use[a]	22.1 ± 7.8	N/A	20.1 ± 1.4
Years of heavy methamphetamine use[e]	7.9 ± 6.8	N/A	9.4 ± 1.4

Abbreviations: BP_{ND}, binding potential relative to nondisplaceable uptake of the radiotracer; N/A, not available. [a]Data presented are mean ± s.e., unless otherwise indicated. [b]Number of days of drug use in the 30 days preceeding study entry. [c]Difference between methamphetamine and control groups with [^{18}F]fallypride BP_{ND} ($P < 0.05$ by Student's *t*-test). [d]Difference between methamphetamine and control groups with [^{18}F]fallypride BP_{ND} ($P < 0.05$ by χ^2 test). [e]Data missing for three subjects; heavy use defined as three times per week or at least one 2-day binges per week.

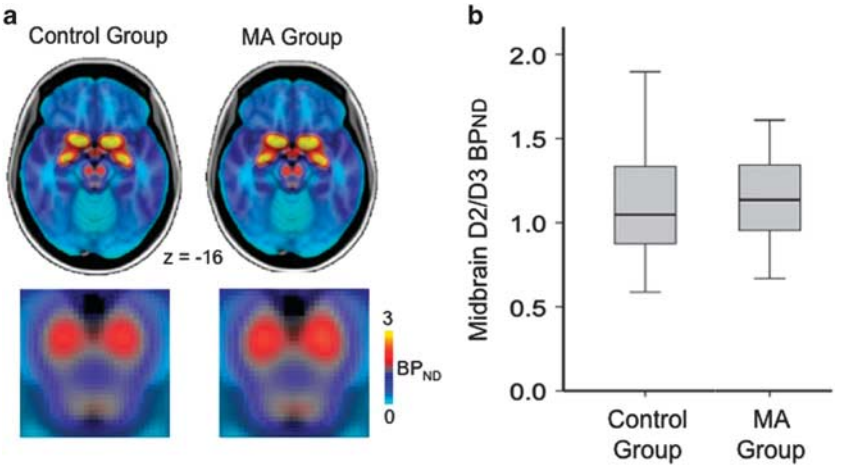

Figure 1. Midbrain D2/D3 BP$_{ND}$ (binding potential relative to nondisplaceable uptake of the radiotracer) in control and methamphetamine (MA) groups. (**a**) In control and MA groups, maps depict average midbrain D2/D3 BP$_{ND}$ across the whole brain with a closer view of the midbrain. The right hemisphere is displayed on the left side of the image. (**b**) Box plots depict average midbrain D2/D3 BP$_{ND}$ in methamphetamine and control groups. Analysis of covariance, with age, sex, frequency of marijuana use and smoking status as covariates, revealed no significant group differences in midbrain D2/D3 BP$_{ND}$.

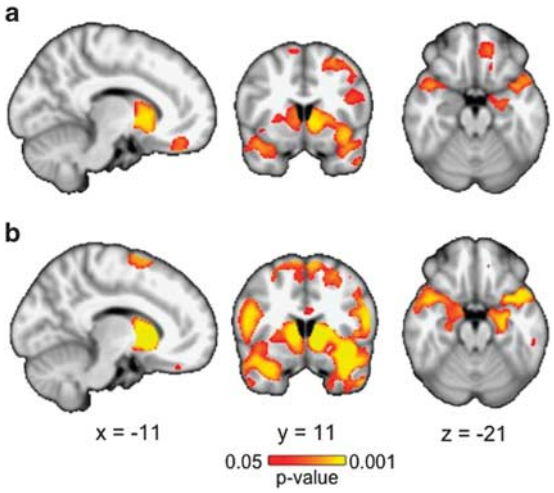

Figure 2. Associations between midbrain D2/D3 BP$_{ND}$ (binding potential relative to nondisplaceable uptake of the radiotracer) and gray-matter volume. (**a**) Statistical maps indicate brain regions where there was a significant group-by-midbrain D2/D3 BP$_{ND}$ interaction on gray-matter volume. Nonparametric analysis of covariance, including age, sex, frequency of marijuana and smoking status as covariates, showed that the relationship between gray-matter volume and midbrain D2/D3 BP$_{ND}$ is greater in methamphetamine users than in controls in the striatum, insula, hippocampus and dorsolateral, orbitofrontal, inferior frontal and temporal cortices ($P < 0.05$, whole-brain correction for multiple comparisons). (**b**) Brain regions where midbrain D2/D3 BP$_{ND}$ was positively associated with gray-matter volume in the methamphetamine group ($P < 0.05$, whole-brain correction for multiple comparisons). See Supplementary Table 1 for complete list of brain regions. The right hemisphere is displayed on the left side of the image.

inferior frontal cortex (Figure 2a and Supplementary Table 1). In the methamphetamine group, midbrain D2/D3 BP$_{ND}$ was positively associated with gray-matter volume in bilateral striatum, amygdala, hippocampus, temporal pole, inferior frontal gyrus, dorsolateral prefrontal cortex and insula (Figure 2b and Supplementary Table 1), whereas no significant associations between gray-matter volume and midbrain D2/D3 BP$_{ND}$ were detected in the control group (P's > 0.4; see Supplementary Figures 2 and 3 for scatter plots and effect size maps).

Midbrain and striatal D2/D3 BP$_{ND}$ were positively correlated in both methamphetamine ($r(31) = 0.63$, $P < 0.001$) and control groups ($r(37) = 0.72$, $P < 0.001$). Therefore, we examined the correlation between striatal D2/D3 BP$_{ND}$ and gray-matter volume across the whole brain. Group-by-striatal D2/D3 BP$_{ND}$ interactions were detected on gray-matter volume of bilateral striatum, left pallidum and temporal cortex (Supplementary Table 2 and Supplementary Figure 4), reflecting positive correlations between striatal D2/D3 BP$_{ND}$ and gray-matter volume in the methamphetamine group, but no significant correlations in the control group. As both striatal and midbrain D2/D3 BP$_{ND}$ were associated with bilateral striatal volume in the methamphetamine group, we examined whether D2/D3 BP$_{ND}$ in each region was uniquely associated with striatal volume in a shared model. In the methamphetamine group, linear regression showed that striatal ($\beta = 0.38$, $t(24) = 3.00$, $P = 0.007$) and midbrain D2/D3 BP$_{ND}$ ($\beta = 0.28$, $t(24) = 2.30$ $P = 0.03$) were both significant predictors of striatal gray-matter volume when included in the same statistical model along with age, gender, smoking status and frequency of marijuana use as covariates (adjusted $R^2 = 0.759$, $F(6,24) = 16.75$, $P < 0.001$; Figure 3). In the control group, striatal gray-matter volume was not associated with striatal ($\beta = -0.18$, $t(30) = -0.68$, $P = 0.50$) or midbrain D2/D3 BP$_{ND}$ ($\beta = -0.02$, $t(30) = -0.07$, $P = 0.95$) in a linear regression that also included age, sex, smoking status and frequency of marijuana use as covariates (adjusted $R^2 = 0.150$, $F(6,30) = 2.01$, $P = 0.09$).

Relationships of methamphetamine craving to dopamine D2/D3 BP$_{ND}$ and gray-matter volume

Self-reported craving was not correlated with age ($r(58) = -0.11$, $P = 0.43$), frequency of alcohol use ($r(58) = -0.005$, $P = 0.97$), number of alcoholic drinks per week ($r(58) = -0.10$, $P = 0.46$), frequency of marijuana use ($r(58) = -0.04$, $P = 0.78$), lifetime cigarette exposure (pack-years) ($r(58) = -0.13$, $P = 0.32$) or years of heavy methamphetamine use ($r(55) = -0.02$, $P = 0.88$). The index, days since last methamphetamine use, was positively associated with craving ($r(58) = -0.36$, $P = 0.006$), and women reported stronger craving than men ($t(56) = -2.19$, $P = 0.03$). Furthermore, there was a trend for those who used methamphetamine more often (days

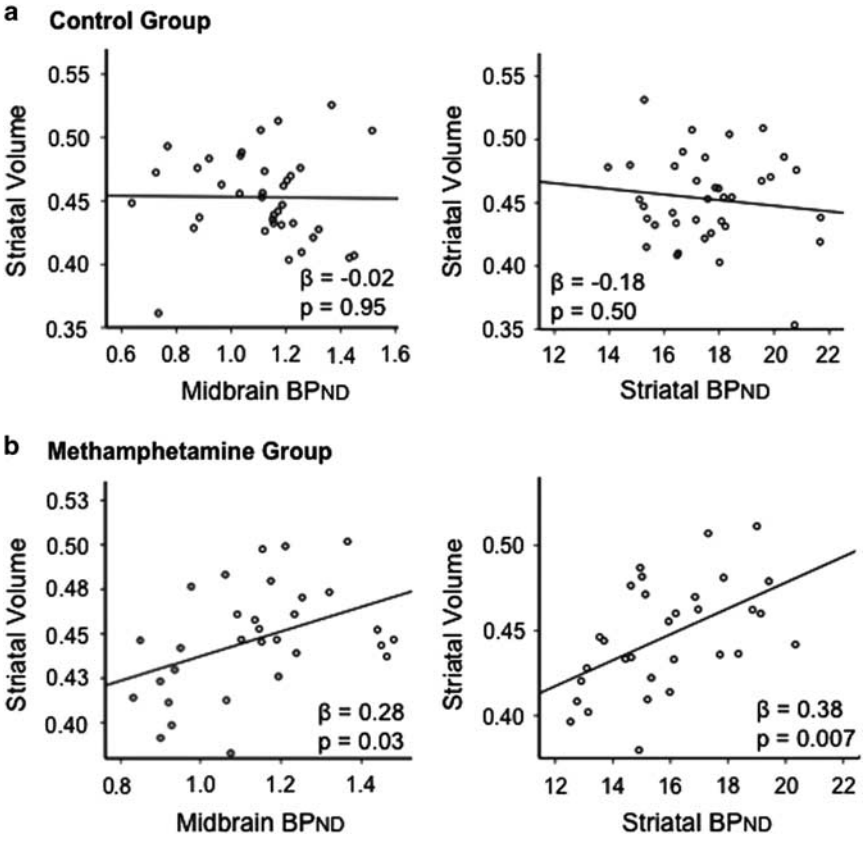

Figure 3. Associations between dopamine D2/D3 BP_{ND} (binding potential relative to nondisplaceable uptake of the radiotracer) and striatal volume. (**a**) Scatter plots show that in the control group, midbrain and striatal D2/D3 BP_{ND} were not associated with striatal volume when included in the same linear regression along with age, sex, frequency of marijuana use and smoking status. (**b**) In contrast, midbrain and striatal D2/D3 BP_{ND} were both associated with striatal volume when included in the same linear regression along with age, sex, frequency of marijuana use and smoking status in the methamphetamine group. Values in the scatter plots are adjusted for all other covariates in the statistical model.

of use in the past 30 days) to report stronger craving ($r(58) = 0.25$, $P = 0.06$); therefore, these variables were included as covariates in statistical analyses examining the relationship of methamphetamine craving to gray-matter volume or dopamine D2/D3 BP_{ND}. No statistically significant relationships between midbrain ($r(24) = 0.01$, $P = 0.95$) or striatal D2/D3 BP_{ND} ($r(24) = -0.12$, $P = 0.55$) and methamphetamine craving were detected ($n = 29$). In contrast, whole-brain voxelwise analysis revealed that methamphetamine craving was negatively correlated with gray-matter volume in bilateral insula, orbitofrontal cortex, rostral anterior cingulate, lateral prefrontal cortex, lateral occipital cortex, parietal cortex, cerebellum and thalamus, and in the left paracingulate gyrus ($P < 0.05$ corrected; Figure 4 and Supplementary Table 3, see Supplementary Figures 5 and 6 for scatter plots and effect size maps).

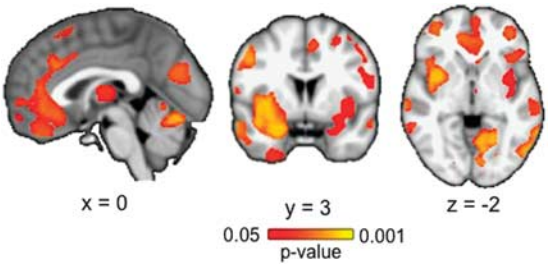

Figure 4. Craving for methamphetamine is negatively correlated with gray-matter volume. Brain regions show where there was a negative association between gray-matter volume and methamphetamine craving. Statistical maps generated using permutation testing with sex, frequency of methamphetamine use and days since last methamphetamine use as covariates ($P < 0.05$, whole-brain correction for multiple comparisons). See Supplementary Table 3 for complete list of brain regions. The right hemisphere is displayed on the left side of the image.

DISCUSSION

This work extends previous findings of structural brain abnormalities associated with chronic methamphetamine use by demonstrating that in methamphetamine users, gray-matter volume varies with midbrain and striatal D2/D3 BP_{ND} and with methamphetamine craving. As the midbrain has a high proportion of dopamine autoreceptors, the positive relationship between midbrain D2/D3 BP_{ND} and gray-matter volume in the methamphetamine group but not in the control group, suggests that capacity for regulating dopamine signaling may influence methamphetamine-induced neurotoxicity in brain regions that receive dopaminergic projections from the midbrain. Methamphetamine-induced toxicity may have a similar influence on both dopaminergic markers and brain morphology in the striatum,[12] consistent with the positive association between striatal D2/D3 BP_{ND} and striatal gray-matter volume detected in

the methamphetamine group, but not in the control group. These associations remained significant in the methamphetamine group after including midbrain D2/D3 BP_{ND} as a covariate, suggesting that additional factors that influence the extent of neurotoxicity or that predate drug use influence the association between striatal BP_{ND} and gray-matter volume in methamphetamine users.

An emerging literature suggests that the regulation of dopamine release, synthesis and firing rate by dopamine D2/D3 autoreceptors[22] has an important role in addiction. For example, mice lacking D2 autoreceptors are more sensitive to the rewarding effects of cocaine than wild-type mice, as indexed by a conditioned place preference procedure.[48] Studies of healthy individuals show that impulsivity and novelty seeking, personality traits associated with vulnerability for addiction,[49–52] are negatively correlated with midbrain D2/D3 BP_{ND}.[21,53]

More work is necessary to determine how methamphetamine exposure influences dopamine receptors in the midbrain. A study in rodents demonstrated that amphetamine self-administration attenuates D2 autoreceptor function.[54] As D2 autoreceptors inhibit the firing of dopamine neurons and dopamine synthesis and release, this finding may help explain why resting-state functional connectivity of the midbrain to the mesocorticolimbic system is greater in methamphetamine users than control participants.[55] Although methamphetamine and control groups did not differ in the midbrain D2/D3 BP_{ND}, it is possible that this finding reflects some nonspecificity of the radiotracer used. [^{18}F]fallypride binds to both D2 and D3 receptors with nearly equal affinity,[56] and a study using the D3 receptor-preferring ligand [^{11}C]-(+)-propyl-hexahydro-naphtho-oxazin showed that D3 receptor availability was higher in the substantia nigra of methamphetamine users than controls.[57] Both D2 and D3 receptors are found in midbrain;[58] therefore, it is possible that D2 receptor deficits in the midbrain are masked by elevated D3 binding.[57]

Although midbrain D2/D3 BP_{ND} was correlated with gray-matter volume in brain regions that are also associated with methamphetamine craving, no direct association between craving and midbrain D2/D3 BP_{ND} was detected. Dopamine release triggered by conditioned cues is correlated with craving;[23,24] however, methylphenidate-induced increases in striatal dopamine do not produce craving unless methylphenidate is paired with drug cues,[59] suggesting that dopamine signaling in the striatum is not sufficient to produce drug craving. Based on studies in animals showing that engagement of frontal–mesencephalic, amydala–mesencephalic and frontal–striatal pathways is necessary for the reinstatement or drug-seeking behavior,[60,61] it has been hypothesized that engagement of these circuits is also important for eliciting drug craving in humans.[59] These findings suggest that assessments of neurochemical signaling from frontal and limbic regions to midbrain and/or striatum may be more useful for predicting individual differences in drug craving.

Previous studies have revealed differences between healthy and methamphetamine-dependent individuals in gray-matter morphometry in striatum, insula and prefrontal cortex;[8–10,62] this study extends these results by linking these deficits to a feature of methamphetamine-use disorder. Methamphetamine craving was negatively correlated with gray-matter volume in a distributed set of brain regions that have consistently been implicated in functional[32] and structural brain studies of craving for other drugs.[63,64] Functional MRI and PET studies show that greater self-reported cue-induced craving is positively correlated with activation in the striatum, and orbitofrontal, dorsolateral prefrontal and anterior cingulate cortices.[32] There are many functional and structural connections between these regions[65] and signaling through these pathways may remain intact or perhaps even heightened in response to drug-related stimuli. In contrast, methamphetamine users display less activation in prefrontal and anterior cingulate cortices than healthy controls while engaging in tasks that require self-control.[66,67] As less self-control is associated with greater methamphetamine craving,[33] future studies might combine structural and functional MRI to determine if deficits in gray-matter volume disrupt functional connectivity in circuitry relevant for self-control and drug craving.

Methodological limitations associated with self-report measurements of craving warrant mentioning. In this study, participants retrospectively rated their craving for methamphetamine in the 24 h before testing; however, little is known about the temporal dynamics of craving.[68] Individuals included in this study were made aware that participation required maintaining abstinence from methamphetamine for at least 7 days; and the circumstances surrounding study participation may have influenced craving levels. It is possible that more naturalistic assessments of methamphetamine craving may produce different results.

This study provides new evidence that midbrain D2/D3 BP_{ND} is related to gray-matter volume in methamphetamine users, suggesting that dopamine autoreceptors may have a role in limiting methamphetamine-induced toxicity; however, many interrelated mechanisms, such as oxidative stress, excitotoxicity, inflammation, mitochondrial function, hyperthermia and disruption of the blood–brain barrier contribute to methamphetamine-induced cell damage.[17,69] More work is necessary to determine how these neurotoxic mechanisms, genetic variability and other environmental factors influence the MRI signal through changes in synaptogensis, dendritic arborization or cell numbers. Identifying the mechanisms that produce abnormalities in gray-matter volume in methamphetamine users may prove useful for developing interventions that lessen symptoms of drug-use disorders, such as craving.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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