

ONLINE FIRST

Evidence for Chronically Altered Serotonin Function in the Cerebral Cortex of Female 3,4-Methylenedioxymethamphetamine Polydrug Users

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Context: MDMA (3,4-methylenedioxymethamphetamine, also popularly known as “ecstasy”) is a popular recreational drug that produces loss of serotonin axons in animal models. Whether MDMA produces chronic reductions in serotonin signaling in humans remains controversial.

Objective: To determine whether MDMA use is associated with chronic reductions in serotonin signaling in the cerebral cortex of women as reflected by increased serotonin_{2A} receptor levels.

Design: Cross-sectional case-control study comparing serotonin_{2A} receptor levels in abstinent female MDMA polydrug users with those in women who did not use MDMA (within-group design assessing the association of lifetime MDMA use and serotonin_{2A} receptors). Case participants were abstinent from MDMA use for at least 90 days as verified by analysis of hair samples. The serotonin_{2A} receptor levels in the cerebral cortex were determined using serotonin_{2A}-specific positron emission tomography with radioligand fluorine 18-labeled setoperone as the tracer.

Setting: Academic medical center research laboratory.

Participants: A total of 14 female MDMA users and 10 women who did not use MDMA (controls). The main exclusion criteria were nondrug-related DSM-IV Axis I psychiatric disorders and general medical illness.

Main Outcome Measures: Cortical serotonin_{2A} receptor nondisplaceable binding potential (serotonin_{2A}BP_{ND}).

Results: MDMA users had increased serotonin_{2A}BP_{ND} in occipital-parietal (19.7%), temporal (20.5%), occipitotemporal-parietal (18.3%), frontal (16.6%), and frontoparietal (18.5%) regions (corrected $P < .05$). Lifetime MDMA use was positively associated with serotonin_{2A}BP_{ND} in frontoparietal ($\beta = 0.665$; $P = .007$), occipitotemporal ($\beta = 0.798$; $P = .002$), frontolimbic ($\beta = 0.634$; $P = .02$), and frontal ($\beta = 0.691$; $P = .008$) regions. In contrast, there were no regions in which MDMA use was inversely associated with receptor levels. There were no statistically significant effects of the duration of MDMA abstinence on serotonin_{2A}BP_{ND}.

Conclusions: The recreational use of MDMA is associated with long-lasting increases in serotonin_{2A} receptor density. Serotonin_{2A} receptor levels correlate positively with lifetime MDMA use and do not decrease with abstinence. These results suggest that MDMA use produces chronic serotonin neurotoxicity in humans. Given the broad role of serotonin in human brain function, the possibility for therapeutic MDMA use, and the widespread recreational popularity of this drug, these results have critical public health implications.

Arch Gen Psychiatry. 2012;69(4):399-409.

Published online December 5, 2011.

doi:10.1001/archgenpsychiatry.2011.156

M MDMA (3,4-METHYLENEDIOXYMETHAMPHETAMINE, also popularly known as “ecstasy”) is a widely-used drug that is linked to neuropsychiatric impairment and neurophysiological alterations in human users.^{1,2} MDMA acutely increases synaptic concentrations of serotonin and other monoamines³ that produce mood changes,⁴ which accounts for its recreational popu-

larity. US surveys indicate that 6% of high school seniors and 5% of the general population report lifetime MDMA exposure.⁵ Across the European Union, the average lifetime MDMA use is 5.6% in young adults, with the highest rates in the United Kingdom (15.4%) and Denmark (10.5%).⁶ Neurotoxic regimens of MDMA (eg, 5 mg/kg of MDMA administered subcutaneously twice a day for 4 days in nonhuman primates⁷ or 40 mg/kg of MDMA administered orally twice a day for 4 days in

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rodents⁸) resulted in the loss of serotonin axons⁹; this loss is greater for the fine diameter cortical serotonin axons that are most distal to the cell bodies of origin. In animals, MDMA spares the cell bodies of brainstem serotonin neurons.⁹ Whether dose regimens that more closely mimic those of humans can lead to loss of serotonin axons is still unknown.

Although the ability of some MDMA dosing regimens to produce chronic serotonin axon loss is clear,⁹ the evidence for MDMA-induced chronic reductions in serotonin signaling in humans remains equivocal.^{1,2} Recent studies suggesting that MDMA enhances psychotherapy¹⁰ and that some MDMA users have minimal impairments in neurocognition¹¹ have potentially enhanced the perception that MDMA is safe for human use. However, there is little evidence for the use of MDMA as a psychotherapeutic agent and a great deal of evidence to support neurocognitive deficits in recreational MDMA users.^{12,13} Given its widespread use, given the efforts to find therapeutic indications, and given the critical role of serotonin in brain function, it is essential to determine whether MDMA use by humans is associated with long-term changes in serotonin function.

Consistent evidence for chronic reductions in serotonin signaling in individuals who use MDMA as a recreational drug derives from nuclear imaging measures of the presynaptic axonal serotonin transporter as a surrogate marker for serotonin axon integrity.¹⁴⁻²¹ The bulk of these studies have found lasting reductions in serotonin transporter binding levels in MDMA users that are prominent in the cerebral cortex.^{1,2} Although reductions in serotonin transporter density are consistent with serotonin axon loss, there is evidence that transporter levels show some recovery with sustained abstinence,^{17,22} suggesting that partial recovery from initial axon injury may be possible.

In contrast to the presynaptic serotonin transporter, the postsynaptic serotonin_{2A} receptor may have advantages as an assay of ongoing serotonin signaling because serotonin_{2A} receptors reflect presynaptic agonist signaling. Serotonin_{2A} receptor levels decrease in the face of increased agonist stimulation,^{23,24} and the release of MDMA-induced serotonin acutely decreases serotonin_{2A} levels in some brain regions of rats.^{24,25} Reductions of 20% to 30% in serotonin markers were associated with reduced serotonin_{2A} binding in cortical and subcortical regions in rats at 3 months after the administration of MDMA, whereas MDMA dosing regimens causing serotonin depletions of around 80% produced chronic increases in serotonin_{2A} binding in the frontal cortex of rats.²⁵ A preliminary study²⁵ of ongoing MDMA users found reduced serotonin_{2A} levels in all brain regions using single-photon emission computed tomography, whereas abstinent MDMA users had increased serotonin_{2A} receptor binding only in the occipital cortex. Erritzoe and colleagues²⁶ examined both serotonin_{2A} receptor binding and serotonin transporter binding in humans who recently used MDMA and hallucinogens (and were characterized as either MDMA-preferring users or hallucinogen-preferring users). Overall, there was a slight tendency for lower serotonin_{2A} receptor levels in the MDMA- and hallucinogen-preferring users than in the controls, but no differences in serotonin_{2A} binding were seen between the 2 drug-using groups, despite widespread reductions in

the serotonin transporter level that were restricted to the MDMA-preferring users.

In addition to the need to determine whether MDMA use is associated with chronic serotonin loss in humans, more evidence is needed regarding the effects of MDMA on women. Sex has been shown to influence toxicity to drugs of abuse.²⁷ Female and male users metabolize MDMA differently,²⁸ with female MDMA users reporting more pronounced subjective effects, such as hallucinogen-like effects.²⁹ Although most studies do not report an association between sex and serotonin transporter levels in MDMA users, a minority of studies reported greater reductions in the serotonin transporter level^{19,22} in female users. Female MDMA users also had lower 5-hydroxyindoleacetic acid (5-HIAA, a serotonin metabolite) levels than did male MDMA users.³⁰ Additionally, serotonin_{2A} receptor binding has also been associated with sex differences, with women having reduced serotonin_{2A} binding levels compared with men.^{31,32} We therefore focused on recruiting female MDMA users.

The evidence to date suggests that human recreational MDMA use may lead to chronic alterations in cortical serotonin function. To determine whether MDMA use is associated with chronic changes in serotonin signaling as reflected by serotonin_{2A} receptor levels, we used fluorine 18 (¹⁸F)-labeled setoperone³³ positron emission tomography (PET) to assay cerebral cortical serotonin_{2A} receptors in long-abstinent female MDMA polydrug users and controls. We estimated receptor levels as the nondisplaceable binding potential (BP_{ND}), which reflects receptors available to bind [¹⁸F]setoperone. In line with the observations of Reneman and colleagues,²⁵ we hypothesized that long-abstinent MDMA users would have increased serotonin_{2A} receptor levels in the cortex and that greater lifetime MDMA exposure would predict greater serotonin_{2A} receptor levels.

METHODS

PARTICIPANTS

A total of 15 female MDMA users and 10 female non-MDMA-exposed controls (both groups 18 to 25 years of age) completed [¹⁸F]setoperone PET scans to determine serotonin_{2A} receptor status in the cerebral cortex. Data from 1 female MDMA user was excluded because it was found by analysis of hair samples that she had recently used cocaine.

We recruited control participants in parallel with MDMA users by advertising in the local media, in flyers, and by word of mouth requesting participants who had used ecstasy, marijuana, or other recreational drugs. Participants were compensated for their time. Our study was approved by the Vanderbilt University institutional review board and conformed to the World Medical Association's Declaration of Helsinki. Participants were screened by telephone for inclusion and exclusion criteria, and those who met the provisional enrollment criteria were additionally screened in person.

INCLUSION CRITERIA

White women 18 to 25 years of age who did not use drugs of abuse within 2 weeks of enrollment were eligible for inclusion in our study. Participants were required to have regular men-

strual cycles or use oral hormonal contraceptives. Control group participants did not use MDMA, whereas MDMA users reported MDMA use on at least 5 occasions (a minimum exposure based on our prior functional magnetic resonance imaging [fMRI] studies³⁴⁻³⁷). For all participants, their last exposure to cocaine, lysergic acid diethylamide, and other amphetamines had to be at least 90 days prior to enrollment in our study. These use and abstinence criteria were chosen on the basis of our earlier fMRI studies and on the basis of the literature reviewed regarding the time frame of serotonin_{2A} receptor changes following MDMA exposure.

EXCLUSION CRITERIA

The exclusion criteria were having general medical conditions or endocrine abnormalities; having contraindications to PET or MRI scanning; having a lifetime history of Axis I psychiatric diagnoses, except for drug-induced mood disorder (1 MDMA user met criteria for a history of substance-induced hypomania); being currently dependent or having been previously dependent on a substance other than nicotine or caffeine; positive urine drug screen within 2 weeks of the PET scan, and alcohol use within 72 hours of the PET scan; use of psychoactive or vasoactive medications within 6 weeks of enrollment; and head injury with loss of consciousness greater than 20 minutes.

SCREENING

Participants were screened with the Mini-International Neuropsychiatric Interview,³⁸ the North American Adult Reading Test (to assess verbal IQ quotient), a urine drug test (Triage Drugs of Abuse Panel; Biosite Diagnostics), a urinary cotinine test (Cotinine Test Device; Innovacon), and a urine pregnancy test (Sure-Vue Urine hCG; Fisher HealthCare). Participants were screened for drug use, cotinine, and pregnancy twice a week for at least 2 weeks and until completing the PET scan. Participants with positive drug or pregnancy test results were excluded from our study. Participants provided their history of drug use in a self-reported drug use questionnaire,^{34,36,37} using a time-line follow-back method.³⁹ Lifetime units of each reported drug were calculated as the product of the lifetime episodes of a drug (defined as use within a single 24-hour period) and the average use per episode. Because the MDMA content of ecstasy pills was unknown, lifetime MDMA exposure was estimated as the amount of ecstasy consumed. Participants also completed personality and psychiatric symptom inventories for exploratory analyses of comorbid conditions potentially increased in ecstasy users; these inventories included assessments for depression (the Beck Depression Inventory^{40,41} and the Hamilton Rating Scale for Depression with 17 variables⁴²), anxiety (the Hamilton Anxiety Scale⁴³), impulsiveness (the Barratt Impulsivity Scale, version 11,⁴⁴ and the Temperament and Character Inventory,⁴⁵ Impulsiveness/novelty seeking subscale 2), and novelty seeking (the Temperament and Character Inventory/novelty seeking total score⁴⁶).

Twenty-four hours prior to PET scanning, we assayed serum estrogen levels. Urine drug and cotinine tests were performed prior to the PET scan for all participants to reconfirm drug abstinence and self-reported nicotine exposure. An alcohol breathalyzer (Intoximeters) was used to confirm recent abstinence from alcohol. A hair sample was obtained to perform a drug analysis of prolonged abstinence (up to 90 days) (United States Drug Testing Laboratories). Hair specimens that are used to measure exposure to MDMA correlate well with self-reports.⁴⁷ The results from the hair analysis lead to the removal of 1 participant who tested positive for cocaine. All other participants tested negative for drug use within the 90-day exposure window.

PET SCANNING

The tracer [¹⁸F]setoperone has been used by a number of investigators to study cortical serotonin_{2A} receptor levels in depression,^{48,49} schizophrenia,^{50,51} and Alzheimer disease⁵² and to measure the effects of antipsychotic and antidepressant medications^{53,54} on serotonin_{2A} receptor levels. The binding of [¹⁸F]setoperone to serotonin₂ receptors in the cortex largely reflects serotonin_{2A} receptors, with there being an affinity for serotonin_{2A} vs serotonin_{2C} receptors of 100:1.¹³ The tracer [¹⁸F]setoperone has a subnanomolar binding affinity for the serotonin₂ receptor ($K_D=0.37\text{nM}$, $K_D=0.7\text{nM}$) and a moderate affinity for the dopamine₂ (D₂) receptor ($IC_{50}=56\text{nM}$, D₂/serotonin₂ K₁ ratio of 70) and α_1 receptor ($IC_{50}=38\text{nM}$, α_1 /serotonin₂ K₁ ratio of 35).^{55,56} In humans, a specific cortical uptake is due to binding to serotonin_{2A} receptors, whereas a striatal uptake is due to binding at both serotonin_{2A} and D₂ receptors.³³ In humans, the tracer [¹⁸F]setoperone has polar radiolabeled metabolites that do not appear to cross the blood barrier, unlike another serotonin₂ ligand (ie, [¹⁸F]altanserin).^{57,58}

The tracer [¹⁸F]setoperone was synthesized using a single-mode microwave accelerator and a modified commercial fluorination module (TRACERlab FX FN; GE Healthcare).⁵⁹ The PET scans were acquired with a GE Discovery LS scanner using a 3-dimensional emission acquisition (with a reconstructed resolution of 5.0-5.5 mm). Serial scans of increasing lengths were started immediately after the intravenous injection of 7.0 mCi of [¹⁸F]setoperone (specific activity greater than 1000 Ci/mmol) during a 20-second period and were obtained for 70 minutes. Eight 15-second scans, six 30-second scans, five 1-minute scans, two 2.5-minute scans, three 5-minute scans, and four 10-minute scans were obtained.

IMAGE AND DATA ANALYSIS

The PET images were preprocessed as previously reported elsewhere.⁶⁰⁻⁶³ The dynamic PET scans were coregistered using an independent implementation of a rigid-body mutual-information algorithm. This algorithm registers images by computing a transformation that maximizes the mutual information between the images.^{64,65} We used a multiresolution algorithm couple with a resampling strategy to avoid local extrema problems reported by Pluim and colleagues⁶⁶ when registering images with the same voxel dimensions. This algorithm is fully automatic and does not require any type of manual preprocessing.

Regional serotonin_{2A}BP_{ND} and parametric images of BP_{ND} were calculated using the full reference region method.⁶⁷ We bilaterally sampled the cerebellum using automated processes to create the reference region. The very low levels of serotonin_{2A} receptors in the cerebellum do not invalidate the use of the cerebellum as a reference region for estimating serotonin₂ receptor levels in the cortex. Blin et al³³ and Petit-Taboué et al⁶⁸ have demonstrated that neocortical-to-cerebellar ratios of [¹⁸F]setoperone uptake at 30 to 60 minutes demonstrate correlation coefficients of 0.97 to 0.99 with modeled estimates using either Logan graphical analysis or compartmental modeling. Using the cerebellum as a reference region, the correlation of estimates of serotonin_{2A} receptor levels obtained with a 4-compartment (3-tissue compartments plus plasma) model with least squares fitting and the reference region approach have shown an $r^2=0.95$ with a 0 intercept.⁶⁹

Data were analyzed using SPM5 (Wellcome Department of Cognitive Neuroscience). Serotonin_{2A}BP_{ND} maps were coregistered to each individual's magnetic resonance high-resolution 3-dimensional anatomical T1-weighted image acquired by the use of Philips Intera Achieva 3-T MRI scanner. Images were spatially normalized into Montreal Neurological

Table 1. Demographics and Drug Use of Abstinent Female MDMA Polydrug Users and Women Who Did Not Use MDMA

	Group, Median (IQR) [Minimum-Maximum Use]		P Value
	Non-MDMA (n = 10)	MDMA (n = 14)	
Age, mean (SD), y	21.60 (1.78)	21.64 (2.17)	.81
NAART full-scale IQ, mean (SD)	108.07 (7.66)	108.13 (7.21)	.82
MDMA (as ecstasy) use			
Days since last dose	...	689.50 (238.50-1188.75) [137.00-1837.00]	
Lifetime episodes	...	13.50 (6.00-26.88) [5.00-375.00]	
Lifetime units, mg	...	1400.00 (337.50-3100.00)	
Other drugs, lifetime units	...		
Alcohol, No. of standard drinks	384.50 (105.00-1768.00) [0.00-6130.00]	1231.25 (423.63-1832.50) [62.00-11000.00]	.38
Cannabis, No. of standard joints	63.10 (10.00-240.00) [0.00-500.00]	122.50 (12.98-1250.00) [2.00-4006.00]	.34
Amphetamine, mg	0.00 (0.00-135.00) [0.00-720.00]	0.00 (0.00-195.00) [0.00-1750.00]	.97
Cocaine, g	0.00 (0.00-0.02) [0.00-21.00]	0.00 (0.00-8.40) [0.00-41.00]	.19
Sedative/hypnotics, mg	0.00 (0.00-55.00) [0.00-200.00]	0.00 (0.00-75.99) [0.00-200.00]	.73
Lysergic acid diethylamide, µg	0.00 (0.00-0.00) [0.00-65.00]	0.00 (0.00-620.00) [0.00-19350.00]	.09
Psilocybin, g	0.00 (0.00-0.00) [0.00-10.00]	11.10 (3.81-17.25) [0.00-200.00]	.001 ^a
Nicotine, mg	0.00 (0.00-0.00) [0.00-459.90]	0.00 (0.00-2874.38) [0.00-20075.00]	.22

Abbreviations: IQR, interquartile range (the 25th-75th IQR represents the middle 50% of the values); MDMA, 3,4-methylenedioxyamphetamine; NAART, North American Adult Reading Test.

^aStatistically significant ($P < .05$, uncorrected).

Institute space using the SPM5 avg152T1 template. Normalized BP_{ND} images were smoothed with an 8-mm full-width at half-maximum Gaussian kernel. The final voxel resolution was 2 × 2 × 2 mm.

Prior reports indicate that use of birth control, estrogen level,⁷⁰ and age⁷¹ affect serotonin_{2A} receptor expression. We used multivariable regression within SPM5 to test for the effects of confounding factors (use of birth control, estrogen level, and age) on serotonin_{2A}BP_{ND}. Use of birth control and estrogen level were significantly positively associated with serotonin_{2A}BP_{ND}, and age was significantly negatively associated with serotonin_{2A}BP_{ND} ($P < .01$, corrected). Therefore, subsequent between- and within-group analyses were adjusted for use of birth control, estrogen level, and age.

Because [¹⁸F]setoperone is not specific to serotonin_{2A} binding outside of the cortex,³³ we used the Wake Forest University Pickatlas tool (version 2.4) in SPM to apply a cortical mask to exclude subcortical regions. Because we had no firm a priori hypotheses regarding which cortical regions would be most strongly affected by MDMA, we chose to perform a whole cortex regression analysis as opposed to a region-of-interest analysis. This approach was also chosen to avoid potential partial volume effects inherent in a region-of-interest analysis if the effect of interest is confined to a portion of the region of interest. To adjust for multiple comparisons in the search volume, we used Monte Carlo simulations implemented in AlphaSim (<http://afni.nimh.nih.gov/>) to generate a familywise error-corrected $P = .05$. This corrected P value corresponded to a voxel level of $P = .05$ and a cluster size of 910 voxels.

BETWEEN-GROUP ANALYSIS

The nonparametric Mann-Whitney U test was used to examine differences in drug use between groups for drugs used (nicotine, alcohol, cannabis, amphetamine, cocaine, sedatives/hypnotics, lysergic acid diethylamide, and psilocybin) by more than 10 participants. General linear modeling analysis was performed within SPM5 to assess between-group differences in serotonin_{2A}BP_{ND} between MDMA users and controls while controlling for use of birth control, estrogen level, and age. A Matlab script (MathWorks Inc) was

used to extract the mean serotonin_{2A}BP_{ND} in clusters showing significant between-group differences for subsequent analysis using SPSS for Windows version 18.0 (SPSS Inc). As within SPM5, data were adjusted for use of birth control, estrogen level, and age. This 2-step approach, first to identify areas differing between groups in serotonin_{2A} binding within SPM and then to extract that data for further analysis, was used to ensure that the association of MDMA use with greater serotonin_{2A} binding potential was not explained by other drug use or other factors, such as the duration of drug abstinence.

WITHIN-GROUP ANALYSIS

Animal studies^{9,72-76} reveal a dose-dependent effect of MDMA on serotonin axon loss, and human fMRI studies³⁴⁻³⁶ reveal dose-dependent effects of MDMA on brain activation. As such, these studies predict that increasing MDMA exposure would be associated with higher levels of serotonin_{2A}BP_{ND} if the serotonin_{2A} receptor reflects the same processes associated with MDMA toxicity in animals and with the altered neurophysiology seen in fMRI studies. Therefore, we used multivariable regression analysis to assess the relationship between lifetime MDMA use in units of milligrams of ecstasy and receptor levels within the MDMA user group. We controlled for confounding factors (age, use of birth control, and estrogen level). Once regions displaying a main effect of lifetime MDMA exposure were identified, BP_{ND} values from clusters with significant associations with lifetime MDMA use were extracted (significant associations were defined as those having a familywise error-corrected $P < .05$, which was achieved using a voxel level of $P = .05$ and a cluster extent threshold of 910 voxels), and further analyses were performed in SPSS as for the between-group results.

RESULTS

Data on the demographics and lifetime drug use of the participants are summarized in **Table 1**. Owing to the skewed distributions of the drug use variables, those distributions are described by the median value and the 25th

to 75th interquartile range representing the middle 50% of the observed value, and by the minimum and maximum values. No statistically significant differences between the groups were observed for age, IQ score, depression, anxiety, impulsivity, or novelty ratings ($P > .05$). Psilocybin was the only drug found to have a significant between-group difference ($P = .001$).

BETWEEN-GROUP ANALYSIS

MDMA users had increased serotonin_{2A}BP_{ND} in 5 cortical clusters (**Figure 1**; **Table 2**) that were mainly localized to the occipitoparietal, temporal, occipitotemporal-parietal, frontal, and frontoparietal regions but also included limbic areas. The greatest between-group difference in serotonin_{2A}BP_{ND} was found in the temporal cluster (**Table 3**), where serotonin_{2A} receptor availability was 20.5% higher in the MDMA users. The other regions showed increases in serotonin_{2A}BP_{ND} of 19.7% (occipitoparietal region), 18.3% (occipitotemporal-parietal region), 16.6% (frontal region), and 18.5% (frontoparietal region) in MDMA users. Regions having increased serotonin_{2A} binding potential involved more brain regions in the right hemisphere than in the left. This was notable for the right frontal regions (Table 2). In contrast to the widespread increases in serotonin_{2A}BP_{ND} in MDMA users, there were no regions in which serotonin_{2A}BP_{ND} was lower in MDMA users than in controls ($P > .05$, corrected).

WITHIN-GROUP ANALYSIS OF MDMA DOSE EFFECTS

Lifetime MDMA use (as ecstasy in units of milligrams) was positively associated with receptor binding in 4 cortical clusters located mainly in the frontoparietal ($\beta = 0.665$, $P = .007$), occipitotemporal ($\beta = 0.798$, $P = .002$), frontolimbic ($\beta = 0.634$, $P = .02$), and frontal regions ($\beta = 0.691$, $P = .008$) (**Figure 2**; **Table 4**). In contrast, there were no regions in which lifetime MDMA use was statistically significantly associated with lower serotonin_{2A} receptor availability. Although serotonin_{2A}BP_{ND} levels were nonsignificantly positively associated with the duration of MDMA abstinence, the duration of abstinence was not statistically significantly associated with serotonin_{2A}BP_{ND} in any region (frontoparietal cluster [$\beta = 0.462$, $P = .34$]; occipitotemporal cluster [$\beta = 0.200$, $P = .97$]; frontolimbic cluster [$\beta = 0.331$, $P = .53$]; and frontal cluster [$\beta = 0.450$, $P = .37$]; $P > .05$, uncorrected) in the model that was adjusted for covariates but that excluded the predictor variable of lifetime MDMA use. When we included the duration of MDMA abstinence in the regression model, the association of lifetime MDMA use remained significant in all regions ($P < .05$). This result suggested that the association between lifetime MDMA use and increased serotonin_{2A}BP_{ND} does not weaken with extended abstinence.

WITHIN-GROUP ANALYSIS OF POLYDRUG USE EFFECTS

To ensure that the observed association of lifetime MDMA use with serotonin_{2A}BP_{ND} was not driven by exposure to

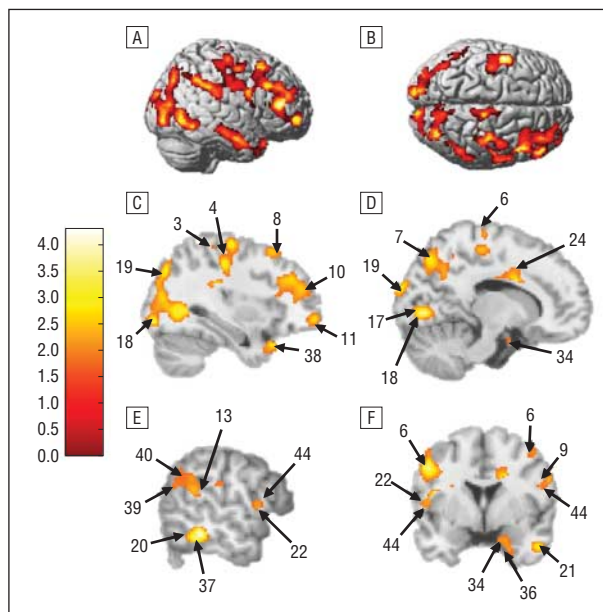


Figure 1. Regions of increased serotonin_{2A} receptor nondisplaceable binding potential (serotonin_{2A}BP_{ND}). A and B, Right hemisphere and bilateral superior regions, respectively, reveal that serotonin_{2A}BP_{ND} was greater in users of 3,4-methylenedioxymethamphetamine (MDMA) than in controls (Table 2). C-F, The Brodmann areas are labeled on brain sections having increased serotonin_{2A}BP_{ND} in MDMA users (after adjusting for age, use of birth control, and estrogen level) in the representative clusters from the right hemisphere (A) and bilateral superior (B) regions. The color scale indicates the *t* score for significant voxels (voxel level, $P = .05$; cluster volume, 910 voxels; familywise error-corrected $P = .05$).

other drugs of abuse, we conducted analyses examining the association of regional serotonin_{2A}BP_{ND} with other drug use, including nicotine. There were no statistically significant associations ($P > .05$, uncorrected) of lifetime units of other drugs with serotonin_{2A}BP_{ND} in any cluster (frontoparietal, occipitotemporal, frontolimbic, or frontal).

COMMENT

The current findings reveal that female MDMA users have chronic changes in cortical serotonin function that consist of greater serotonin_{2A} receptor levels and increasing serotonin_{2A} receptor levels with increasing MDMA use. We hypothesized that MDMA users would have increased cortical serotonin_{2A}BP_{ND}, a finding consistent with MDMA-induced chronic reductions in cortical serotonin synaptic neurotransmission leading to compensatory upregulation of the serotonin_{2A} receptors. Although the results support our hypothesis, the interpretation of these findings must remain speculative because we did not measure brain serotonin levels and because factors other than MDMA-induced serotonin axon loss may account for the current findings.

Although the current observations are consistent with chronically reduced serotonin levels in the cerebral cortex, this interpretation is limited by the fact that the serotonin_{2A} receptor has not been sufficiently validated as a measure of serotonin denervation, and it is also possible that chronic reductions in cortical serotonin neurotransmission might occur in the absence of serotonin

Table 2. Between-Group Analysis of Abstinent Female MDMA Polydrug Users and Women Who Did Not Use MDMA^a

Cluster (Volume) and Lobe	Brodman Area(s)	Gyrus/Region
Occipitoparietal (29 192 mm ³)		
Occipital	17, 18, 19	R middle occipital, B cuneus, R superior occipital, R lingual, R calcarine, R inferior occipital
Parietal	7, 31	B precuneus, R superior parietal, R angular
Temporal	37	R inferior temporal, R middle temporal, R fusiform
Temporal (8368 mm ³)		
Temporal	20, 21, 28, 34, 38	R middle temporal, R superior temporal, R inferior temporal, R fusiform, R superior temporal pole, R middle temporal pole
Limbic	...	R uncus, R parahippocampal, R amygdala
Occipitotemporal-parietal (25 832 mm ³)		
Occipital	17, 18, 19	L cuneus, L middle occipital, L superior occipital, L calcarine, L lingual
Temporal	13, 20, 37	L middle temporal, L inferior temporal, L superior temporal
Parietal	39, 40	L inferior parietal, L supramarginal, L angular, L precuneus, L Rolandic operculum
Frontal	...	L postcentral
Limbic	...	L parahippocampal, L hippocampus, L posterior cingulate
Frontal (8840 mm ³)		
Frontal	4, 6, 9, 44	L precentral, L postcentral, L middle frontal, L inferior frontal, L inferior frontal operculum
Parietal	3	L Rolandic operculum
Temporal	...	L superior temporal
Frontoparietal (40 432 mm ³)		
Frontal	4, 6, 8, 9, 10, 44, 46	R middle frontal, R precentral, R inferior frontal, R superior frontal, R medial frontal, R supplementary motor area, R middle frontal orbital, R inferior frontal orbital, R inferior frontal operculum
Parietal	2, 3, 40	R postcentral, R inferior parietal, R supramarginal, R angular, R paracentral lobule
Limbic	24	R Rolandic operculum, R cingulate
Temporal	...	R superior temporal

Abbreviations: B, bilateral; L, left; MDMA, 3,4-methylenedioxymethamphetamine; R, right.

^aSerotonin_{2A} receptor nondisplaceable binding potentials were adjusted for estrogen level, use of birth control, and age in SPM5. Significant clusters were defined as those having a voxel level of *P* = .05, a cluster volume of 910 voxels, and a familywise error-corrected *P* = .05.

Table 3. Adjusted Mean Serotonin_{2A} Receptor Nondisplaceable Binding Potentials From Between-Group Analysis

Cluster ^a	Group, Mean (SD)		<i>P</i> Value
	Non-MDMA (n = 10)	MDMA (n = 15)	
Occipital-parietal	134.26 (15.66)	160.66 (15.62)	.001
Temporal	118.26 (17.12)	142.45 (17.07)	.003
Occipitotemporal-parietal	124.97 (12.48)	147.90 (12.45)	<.001
Frontal	131.25 (14.27)	153.08 (14.22)	.002
Frontoparietal	119.91 (16.35)	142.09 (16.31)	.004

Abbreviation: MDMA, 3,4-methylenedioxymethamphetamine.

^aAll clusters were derived from images thresholded at an uncorrected voxel level of *P* = .05 and a cluster volume of 910 voxels, to yield a familywise error-corrected *P* = .05. Binding potential values are adjusted for age, use of birth control, and estrogen level.

axon loss. Increased serotonin_{2A}BP_{ND} could theoretically be due to a combination of compensatory receptor upregulation and reduced competition for synaptic serotonin with [¹⁸F]setoperone. However, acute tryptophan depletion has been associated with decreased serotonin_{2A} receptor binding in humans in broad regions of the cortex using [¹⁸F]setoperone⁷⁷ and in the frontal cortex using the highly selective serotonin_{2A} ligand carbon 11-labeled MDL100907.⁷⁸ Studies examining the effects of chronically reduced serotonin levels on the serotonin_{2A}

receptor have produced mixed results; for example, 3 weeks of chronic tryptophan depletion can lead to increased serotonin_{2A} receptor levels in the rat cortex,⁷⁹ and the number of serotonin_{2A} receptors increased in the rat prefrontal cortex 20 days after 5,7-dihydroxytryptamine (5,7-DHT) lesions were identified in the dorsal raphe,⁸⁰ but the number decreased in the cortex 3 weeks after 5,7-DHT lesions were identified in the dorsal and median raphe⁸¹ and remained unchanged in rat frontal cortex 2 weeks after intracisternal 5,7-DHT was identified.⁸² Rats given neurotoxic regimens of MDMA had decreased serotonin_{2A} binding in the frontal, parietal, and occipital cortices at 6 hours after MDMA administration, but at 3 days, the number of receptors decreased only in the occipital cortex.²⁵ Animals studied at 30 days after receipt of MDMA had significant increases in serotonin_{2A} receptor binding only in the frontal regions, despite a 90% average reduction in cortical serotonin and 5-HIAA content.²⁵ The strong relationship between increased MDMA exposure and greater serotonin_{2A}BP_{ND} observed in the present study is in line with animal studies of graded increases in toxicity with increasing exposure to MDMA^{9,73} and is also consistent with results indicating that lifetime MDMA exposure is positively correlated with task-evoked brain activation.³⁴⁻³⁶ MDMA use is also associated with altered functional connectivity⁸³ that is suggestive of reduced serotonin function.³¹ This suggests the possibility that both serotonin_{2A} receptor lev-

els and increased task-evoked activation reflect reduced cortical serotonin signaling.

Reneman and colleagues²⁵ reported that MDMA users abstinent from MDMA for an average of 3.3 weeks had reduced serotonin_{2A} receptor levels in the frontal, parietal, and occipital cortices (as measured by iodine 123–labeled R91150 single-photon emission computed tomography), whereas those abstinent for an average of 19.6 weeks had significantly increased serotonin_{2A} binding in the occipital regions but not the remainder of the cortex. Our findings are consistent with those of Reneman and colleagues²⁵ regarding increased serotonin_{2A} receptor levels in the occipital cortex of abstinent MDMA users; however, our study of a cohort of female MDMA users extends the findings to multiple cortical regions, demonstrates that there is a lack of recovery with extended abstinence, and strengthens the argument for specificity to MDMA exposure by demonstrating a dose-response effect. Several factors (including a homogenous female cohort, verified abstinence, improved kinetic properties and tracer kinetic modeling, and possible increases in resolution [although partially offset by the degree of smoothing] afforded by PET) may have permitted us to detect broader effects. A recent report²⁶ used [¹⁸F]altanserin to examine the serotonin_{2A} receptor levels and carbon 11–labeled 3-amino-4-[2-(di(methyl)amino)methyl]phenyl]sulfanylbenzotrile ([¹¹C]DASB) to examine the serotonin transporter levels in MDMA-preferring and hallucinogen-preferring drug users and controls. Because both hallucinogens and MDMA have serotonin_{2A} agonist effects, Erritzoe and colleagues²⁶ predicted that the serotonin_{2A} receptor levels would be lower in recent hallucinogen and MDMA users than in controls. However, use of MDMA or hallucinogens was not clearly associated with lower serotonin_{2A} receptor binding, despite reductions in the serotonin transporter binding in the MDMA-preferring group. In a subset analysis of users with longer periods of MDMA abstinence that more closely paralleled those of the users in the study by Reneman and colleagues,²⁵ Erritzoe and colleagues²⁶ were unable to detect chronic increases in serotonin_{2A} binding, and they considered that this was potentially due to the opposing effects of hallucinogen use on receptor regulation or to the reduced serotonin transporter binding and associated changes in serotonin transmission. Concern for the possibility of residual acute effects of recent MDMA use led to our choice of a minimum 90-day abstinence from MDMA and other drugs potentially influencing the serotonin_{2A} receptor levels as a requirement for enrollment. Although we did not find evidence to suggest that receptor levels decrease with increasing abstinence from MDMA, it is possible that the serotonin_{2A} receptor levels might have normalized in our cohort with a greater period of abstinence.

An increased serotonin_{2A}BP_{ND} in association with MDMA use was not found in all cortical regions and involved more regions in the right hemisphere. The basis for the pattern of findings is unclear. It is possible that serotonin axons in some brain regions are more vulnerable to the effects of MDMA or that regional differences in serotonin_{2A} receptors account for the findings. There was considerable overlap between regions having increased serotonin_{2A} levels in MDMA users and regions showing a positive association of serotonin_{2A}BP_{ND} and life-

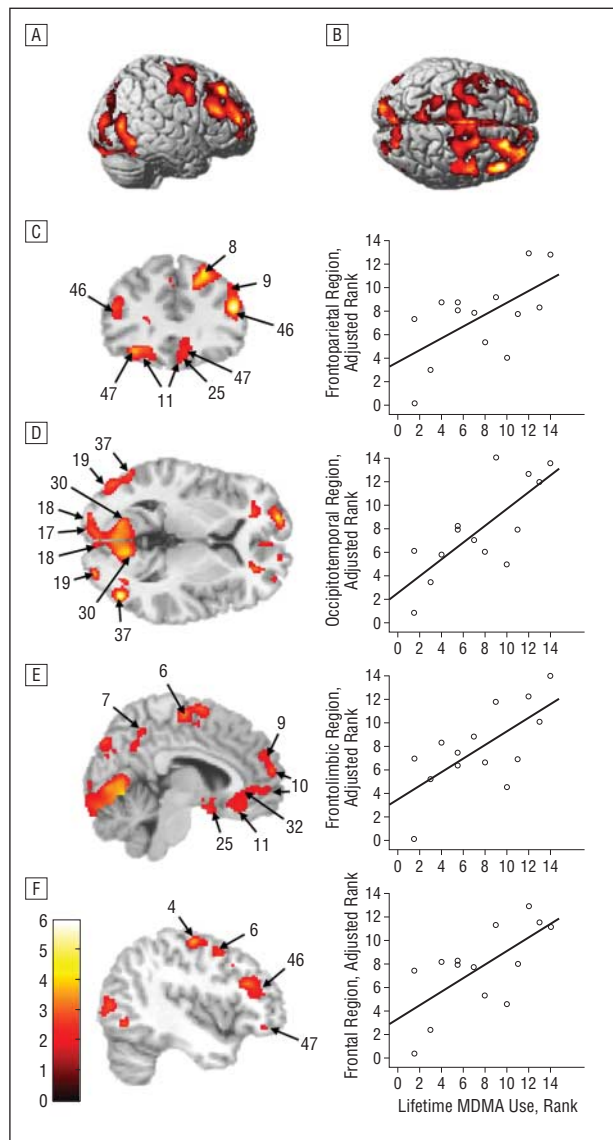


Figure 2. Right hemisphere (A) and bilateral superior (B) regions in which lifetime use of 3,4-methylenedioxymethamphetamine (MDMA) correlates positively with the serotonin_{2A} receptor nondisplaceable binding potential (serotonin_{2A}BP_{ND}) in the MDMA user group after adjusting for use of birth control, estrogen level, and age (Table 4). C-F, The Brodmann areas are labeled on brain sections (left) and scatterplots (right) showing correlation of lifetime MDMA use with serotonin_{2A}BP_{ND}. The color scale indicates the *t* score for significant voxels in the regression analysis (voxel level, *P* = .05; cluster volume, 910 voxels; familywise error–corrected *P* = .05). Scatterplots are rank data to account for the nonparametric distribution of lifetime MDMA use (x-axis; minimum–maximum use, 250–112 500 mg) and the adjusted predicted rank data for serotonin_{2A}BP_{ND} (y-axis; adjusted for age, use of birth control, and estrogen level and using fluorine 18–labeled setoperone as the tracer). The rank of lifetime use was calculated as the product of the average use per episode and the number of use episodes. Ranks were determined within SPSS using the “rank” function.

time ecstasy use, suggesting that MDMA exposure may account for the findings in both analyses.

Although we did not detect differences in depression or anxiety,^{84,85} impulsivity, or novelty seeking between MDMA users and controls, the cross-sectional study method that we used cannot rule out the possibility that preexisting differences in brain function, personality, or behavior^{86,87} might predispose to MDMA use and might also be associated with altered serotonin function. Some

Table 4. Within-Group Regression Analysis of Abstinent Female MDMA Polydrug Users^a

Cluster (Volume) and Lobe	Brodman Area(s)	Gyrus/Region
Frontoparietal (62 984 mm ³)		
Frontal	4, 6, 8, 9, 10, 11, 46	R middle frontal, B medial frontal, B superior medial frontal, R medial frontal orbital, R superior frontal, B precentral, B supplementary motor area, B inferior frontal gyrus, R superior frontal orbital, R inferior frontal operculum, R gyrus rectus
Parietal	2, 3, 5, 7	B precuneus, L postcentral, L paracentral lobule, L superior parietal, B middle cingulate, B anterior cingulate
Occipitotemporal (52 272 mm ³)		
Occipital	17, 18, 19	B lingual, B cuneus, B middle occipital, B calcarine, B superior occipital, B inferior occipital
Temporal	37	B middle temporal, B inferior temporal, B fusiform
Parietal	7, 39	B precuneus, R superior parietal
Limbic	23, 30, 31	B posterior cingulate, B parahippocampal
Frontolimbic (21 544 mm ³)		
Frontal	9, 10, 11, 46, 47	L middle frontal, L superior frontal, B medial frontal, B inferior frontal orbital, B superior frontal orbital, L inferior frontal, L superior medial frontal, L middle frontal orbital, L medial frontal orbital, L gyrus rectus, R insula
Limbic	25	R cingulate, B anterior cingulate, B subcallosal, B parahippocampal, R amygdala
Frontal (7584 mm ³)		
Frontal	4, 6, 8, 9	L precentral, L middle frontal, L superior frontal, L inferior frontal, L postcentral, L inferior frontal operculum

Abbreviations: B, bilateral; L, Left; MDMA, 3,4-methylenedioxymethamphetamine; R, right.

^aSerotonin_{2A} receptor nondisplaceable binding potentials were adjusted for estrogen level, use of birth control, and age in SPM5. Significant clusters were defined as those having a voxel level of $P = .05$, a cluster volume of 910 voxels, and a familywise error-corrected $P = .05$.

evidence suggests that memory impairment persists in abstinent MDMA users, despite the potential for recovery of the serotonin transporter.⁸⁸ This suggests that the persistent changes in the serotonin receptor may be related to chronic memory impairment. However, the relationship between memory, the serotonin_{2A} receptor, and the serotonin transporter may prove complex because McCann and colleagues⁸⁹ reported that verbal memory correlated more strongly with serotonin transporter levels in controls than in MDMA users and because preliminary data suggested that verbal memory was negatively correlated with serotonin_{2A} receptors in MDMA users.¹⁵ The significance of altered serotonin signaling in the absence of detected negative consequences of MDMA exposure remains uncertain, but one possibility is that MDMA users may have compensated for partial reductions in brain serotonin signaling. Because MDMA users have been reported to have impairments in neurocognition,¹² sleep,⁹⁰ and altered pain processing,⁹¹ as well as increased sleep apnea,⁹² studies assessing the relationship of serotonin function with these conditions are warranted.

We applied strict inclusion criteria to our cohort and restricted our study to female users to maximize cohort homogeneity and to isolate the effects of MDMA on serotonin_{2A} receptors. We controlled for factors associated with receptor binding in our cohort (estrogen level, use of birth control, and age), but the number of variables that we adjusted for was large relative to our sample size. Therefore, we may not have fully accounted for the influence of these variables in the overall model. Because we studied healthy women, we cannot generalize these findings to men or individuals with anxiety or depression. However, we consider it likely that similar processes operate in men and that the association of MDMA use with serotonin_{2A} levels is likely

to be equally evident, if not more so, in those more vulnerable to psychiatric disorders. Given the major role that serotonin plays in anxiety and mood disorders, coupled with evidence from some studies that MDMA users have higher rates of anxiety and depression,^{84,85} additional studies that examine serotonin_{2A} receptor status in a broader range of cohorts are warranted. Although we did not find strong evidence that drug use was higher in the MDMA users, this is likely in part due to sample size limitations. However, we did not detect a significant association between other drug use and serotonin_{2A} receptor levels, and it therefore seems unlikely that other drug exposure accounts for the current findings. We did not assess MDMA users to determine why they stopped using MDMA, creating the possibility that we recruited a group of participants who stopped using MDMA owing to negative effects and who potentially were more vulnerable to toxicity. Although we excluded participants who had been diagnosed and treated for psychiatric conditions such as depression, it is possible that some participants had prior exposure to non-recreational drugs that could potentially affect the serotonin_{2A} receptor levels. Although we interpret our findings as consistent with reduced cortical serotonin signaling, we did not obtain additional measures of cortical serotonin signaling, such as cerebrospinal fluid serotonin metabolite levels.^{30,93}

MDMA is a fascinating drug that acutely affects psychological processes and social behaviors. Acute MDMA use produces a sense of improved mood and well-being⁹⁴ and enhances sociability.^{4,95} However, MDMA may also prove to be neurotoxic when taken in large or in multiple dosages,⁹⁶ or when combined with activities (such as dancing) that raise body temperature. Human MDMA plasma concentrations increase greatly when multiple dos-

ages are taken within a narrow time window,⁹⁶ and animal studies have demonstrated that MDMA's serotonin toxicity is dependent on the dose and the presence of hyperthermia.⁹ If MDMA is approved as a treatment for psychiatric conditions, studies determining the dose and conditions that are therapeutic vs neurotoxic will be essential.

In conclusion, our findings indicate that human MDMA use is associated with long-lasting changes in serotonin_{2A} receptor availability that do not decrease with drug abstinence. Our results suggest that MDMA produces chronic alterations in cortical serotonin signaling that is possibly reflective of MDMA-induced neurotoxicity in humans. Given the broad role that serotonin plays in human brain function, the possibility for therapeutic MDMA use, and the widespread recreational popularity of this drug, our results have critical implications for human MDMA users.

Submitted for Publication: May 5, 2011; final revision received August 23, 2011; accepted October 12, 2011.

Published Online: December 5, 2011. doi:10.1001/archgenpsychiatry.2011.156

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Financial Disclosure: None reported.

Funding/Support: This work was supported by the National Institutes of Health with grants R01 DA01537 and R21 DA020149 to Dr Cowan and grant K12 DA00357 to Dr Benningfield (National Institute on Drug Abuse); grant K01 MH083052 to Dr Blackford and grant R21 MH087803-02 to Dr Salomon (National Institute of Mental Health); and Vanderbilt Clinical and Translational Science Award UL1 RR024975 (National Center for Research Resources).

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