Title

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Journal

JAMA Psychiatry, 68(6)

ISSN

2168-622X

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Publication Date

2011-06-06

DOI

10.1001/archgenpsychiatry.2011.56

Peer reviewed

ORIGINAL ARTICLE

In Vivo Imaging of Cerebral Serotonin Transporter and Serotonin$_{2A}$ Receptor Binding in 3,4-Methylenedioxymethamphetamine (MDMA or "Ecstasy") and Hallucinogen Users

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Context: Both hallucinogens and 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") have direct agonistic effects on postsynaptic serotonin$_{2A}$ receptors, the key site for hallucinogenic actions. In addition, MDMA is a potent releaser and reuptake inhibitor of presynaptic serotonin.

Objective: To assess the differential effects of MDMA and hallucinogen use on cerebral serotonin transporter (SERT) and serotonin$_{2A}$ receptor binding.

Design: A positron emission tomography study of 24 young adult drug users and 21 nonusing control participants performed with carbon 11 (^{11}C)–labeled 3-amino-4-[2-[(di(methyl)amino)methyl]phenyl]sulfanylbenzonitrile (DASB) and fluorine 18 (^{18}F)–labeled altanserin, respectively. Scans were performed in the user group after a minimum drug abstinence period of 11 days, and the group was subdivided into hallucinogen-preferring users (n=10) and MDMA-preferring users (n=14).

Participants: Twenty-four young adult users of MDMA and/or hallucinogenic drugs and 21 nonusing controls.

Main Outcome Measures: In vivo cerebral SERT and serotonin$_{2A}$ receptor binding.

Results: Compared with nonusers, MDMA-preferring users showed significant decreases in SERT nondisplaceable binding potential (neocortex, –56%; pallidostriatum, –19%; and amygdala, –32%); no significant changes were seen in hallucinogen-preferring users. Both cortical and pallidostriatal SERT nondisplaceable binding potential was negatively correlated with the number of lifetime MDMA exposures, and the time of abstinence from MDMA was positively correlated with subcortical, but not cortical, SERT binding. A small decrease in neocortical serotonin$_{2A}$ receptor binding in the serotonin$_{2A}$ receptor agonist users (both user groups) was also detected.

Conclusions: We found evidence that MDMA but not hallucinogen use is associated with changes in the cerebral presynaptic serotonergic transmitter system. Because hallucinogenic drugs primarily have serotonin$_{2A}$ receptor agonistic actions, we conclude that the negative association between MDMA use and cerebral SERT binding is mediated through a direct presynaptic MDMA effect rather than by the serotonin$_{2A}$ agonistic effects of MDMA. Our cross-sectional data suggest that subcortical, but not cortical, recovery of SERT binding might take place after several months of MDMA abstinence.

Arch Gen Psychiatry. 2011;68(6):562-576

MDMA (3,4-METHYLENEdioxymethamphetamine), or "ecstasy" and hallucinogens are recreational drugs that frequently are used in the Western hemisphere. During the past 5 years, their use has been relatively stable.[1,2] Currently, the prevalence of MDMA and lysergic acid diethylamide (LSD) use among US children in grades 8 to 12 is 4% and 3%, respectively,[1] and it is 6% and 4% among 15-to 34-year-old Europeans.[2] The term *hallucinogen* refers to a large number of drugs with psychopharmacologic resemblance to either the natural products psilocybin and the semisynthetic substance LSD (tryptamines) or the active component of the peyote cactus, mescaline (phenethylamines). These drugs induce states of altered perception, thought, and feelings not normally experienced except perhaps in dreams. By contrast, users of MDMA maintain a sense of contact with reality, although visual hallucinations may occur.[3]

Both hallucinogens and MDMA exert their primary effects through actions on serotonergic neurotransmission. Multiple pharmacologic actions on the neu-

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ronal serotonergic terminal and synapse occur with MDMA, which is a substrate of the serotonin transporter (SERT) by which it enters the neurons and releases serotonin (5-hydroxytryptophan) from the storage vesicles and by reversal of normal SERT function. In addition, MDMA inhibits tryptophan hydroxylase, the rate-limiting enzyme for serotonin synthesis, and partially inhibits serotonin degradation by monoamine oxidase B.[4] Finally, MDMA has serotonin$_{2A}$ receptor agonist action.[5] This last effect is shared with the hallucinogens, which potently stimulate the serotonin$_{2A}$ receptor, and the serotonin$_{2A}$ receptor agonistic properties are responsible for the behavioral effects of hallucinogens.[6]

Serotonin transporter is crucial for the regulation of serotonin transmission because it controls the levels at the site of the postsynaptic receptors by reuptake of synaptically released serotonin. Some authors consider SERT to be a marker of the integrity of serotonin neurons.[7] Animal[8-16] and human[17-21] studies have firmly established that long-term exposure to moderate and high doses of MDMA is associated with a reduction in cerebral serotonin levels and a decreased number of SERT binding sites. Histologic studies[22] in animals have shown that large doses of MDMA are associated with neurodegeneration particularly affecting the terminal portions of axons; fibers and raphe cell bodies are spared. However, there is no firm evidence of MDMA-induced neurodegeneration in humans.[23,24] By contrast, although the use of hallucinogens is illicit and, in rare cases, can cause persistent false perceptions or flashbacks,[25] these drugs do not cause dependence, and use of hallucinogens is generally considered to be physiologically safe.[26-28] This is most likely why there are few studies on the neurobiologic implications of hallucinogen use.

Several animal studies[29,30] point to a key role of the serotonin$_{2A}$ receptor in MDMA-induced effects on neurons, possibly through activation of the phospholipase A_2 apoptosis pathway. This observation is supported by the notion that pretreatment with serotonin$_{2A}$ receptor antagonists prevents the hyperthermia associated with MDMA use,[31,32] which is considered a key factor in the deleterious effects of MDMA on the brain. In addition, current MDMA users are reported[33] to have decreased cortical serotonin$_{2A}$ receptor binding in contrast to former users, who have increased binding of this receptor similar to that seen in rats. Furthermore, systemic administration of serotonin$_{2A}$ receptor agonists in animals is associated with inhibited firing of raphe serotonin neurons.[34] Because decreased SERT binding has been observed after pharmacologically induced chronic extracellular serotonin depletion in some[35,36] although not all[37] studies, SERT binding may decrease in response to the stimulatory effects on the serotonin$_{2A}$ receptor. Thus, as with MDMA, hallucinogens could affect the availability of both the serotonin$_{2A}$ receptors and SERT.

In this study, we assessed the differential effects of MDMA and hallucinogen use on cerebral SERT and serotonin$_{2A}$ receptor levels by investigating, with use of positron emission tomography (PET), in vivo SERT and serotonin$_{2A}$ receptor binding. Participants included individuals with recreational use of MDMA and hallucinogens and a group of age- and sex-matched healthy individuals who do not use those drugs. To our knowledge, this is the first study to image in vivo cerebral serotonergic markers in recreational users of hallucinogens, as well as the first study in which relevant presynaptic and postsynaptic markers are assessed simultaneously within the same MDMA-using individuals. In comparison with the control group, we expected SERT binding to be decreased only in MDMA users and serotonin$_{2A}$ receptor binding to be decreased in both user groups. For both measured serotonergic markers, we expected that the decrease in SERT binding would be most pronounced in users with a short period of abstinence.

METHODS

RECRUITMENT AND INCLUSION

Participants were recruited by fliers and advertisements posted on relevant Web sites. Some individuals were recruited by word-of-mouth from other participants. Those who appeared to meet inclusion criteria were invited to a face-to-face screening that involved assessment of history of alcohol, tobacco, and illegal drug use (using the Copenhagen Substance Screening Questionnaire [available from the authors on request] and modified Danish versions of the Customary Drinking and Drug Use Record[38] and Lifetime Drinking History[39]), as well as screening of current and previous psychiatric symptoms using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN 2.1) interview.[40]

Individuals between 18 and 35 years old who had a minimum of 12 lifetime exposures to MDMA or hallucinogens, as well as use of MDMA and/or hallucinogens within the year before the scan, were candidates for inclusion. To avoid acute drug effects, including drug receptor/transporter drug occupancy, use of drugs was not allowed for 7 days before the scan. Control individuals were excluded if they reported more than 15 lifetime exposures to cannabis or had any history of other illegal drug use. Individuals with prior neurologic or psychiatric disorders (*ICD-10* or *DSM-IV* Axis I diagnostic criteria for obsessive-compulsive disorder, anxiety, major depression, bipolar disorder/mania, or schizophrenia as assessed with the SCAN 2.1 interview) were excluded. All participants had normal results of a neurologic examination and had never used antidepressants or antipsychotics.

The study was approved by the local ethics committee of Copenhagen and Frederiksberg, Denmark, and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

PARTICIPANTS AND DRUG USE

Twenty-four young adult users of MDMA and/or hallucinogens (21 men and 3 women) with a mean (SD) age of 24.6 (4.0) years and 21 controls (ie, nonusers; 17 men and 4 women) with a mean age of 24.0 (3.4) years were included in the study. As listed in **Table 1**, 1 drug user had never taken MDMA and 3 others had never used hallucinogens. The hallucinogens used by the participants are listed in **Table 2**. Because use of MDMA in tablet form and as a powder was reported, the quantity of MDMA in powder form was translated into a number of ecstasy tablets by dividing the reported number of milligrams per session of MDMA use in powder form by 60. This factor was used because Danish ecstasy tablets, on average, contained 60 mg of MDMA during the study period.[41] Details about drug use, including the calculated lifetime number of consumed ecstasy tablets, are listed in Table 1.

Table 1. Differences in Illegal Drug Use Between MPUs and HPUs

	Group				
	MPU (n=14)		HPU (n=10)		
	Mean (SD) [Median]	Range (IQR)	Mean (SD) [Median]	Range (IQR)	*P* Value[a]
MDMA					
No. of subjects who have ever used	14/14		9/10		
Age at first use, y	19.4 (3.8)	16-31	18.2 (2.2)	14-22	.39
Time since last use, d	57 (62) [44]	11-258 (20-66)	122 (96) [96]	30-293 (73-113)	.012[b]
Length of regular use, mo	72.1 (39.1)	25-145	57.1 (31.7)	25-106	.32
Frequency of use, d/mo	3.3 (2.7)	0.4-9.6	0.3 (0.4)	0.1-1.5	.001
Tablets used in typical session, No.	4.3 (2.7)	1.5-10	1.8 (1.6)	1-5	.02
Powder used in typical session, mg	268 (144)	100-600	143 (56)	50-250	.005
Lifetime sessions with use, No.	236 (204) [180]	13-670 (110-327)	18 (22) [11]	3-74 (8-20)	<.001[b]
Calculated lifetime tablets used, No.	1296 (1801) [635]	33-6590 (317-1172)	60 (114) [24]	6-362 (10-38)	<.001[b]
Hallucinogen					
No. of subjects who have ever used	11/14		10/10		
Time since last use, d	535 (806) [184]	24-1010 (121-595)	35 (26) [26]	14-105 (16-38)	<.001[b]
Age at first use, y	18.0 (2.6)	15-23	18.2 (2.4)	15-23	.86
Length of regular use, mo	71.2 (43.1)	6-145	61.3 (35.0)	25-130	.57
Frequency of use, d/mo	0.38 (0.37)	0.0-1.1	1.7 (1.2)	0.5-4.3	.005
Lifetime sessions with use, No.	23.1 (24.6) [13]	1-80 (8-31)	106.7 (84.2) [107]	21-262 (30-142)	.004[b]
Cannabis					
No of subjects who have ever used	14/14		10/10		
Time since last use, d	523 (1157) [32]	8-3948 (14-266)	43 (66) [21]	10-228 (14-35)	.45[b]
Age at first use, y	15.0 (3.2)	12-25	16.1 (1.5)	14-18	.27
Frequency of use in past 3 mo, d/mo	5.6 (6.2)	0-16	7.1 (7.9)	0-20	.63
Amphetamine					
No. of subjects who have ever used	14/14		7/10		
Time since last use, d	108 (187) [51]	7-710 (16-57)	683 (594) [661]	39-1630 (221-939)	.02[b]
Age at first use, y	18.7 (4.1)	15-31	16.6 (2)	14-20	.12
Frequency of use in past 3 mo, d/mo	1.6 (2.0)	0-5.3	0.3 (0.6)	0-1.7	.04
Cocaine					
No of subjects who have ever used	14/14		7/10		
Time since last use, d	142 (162) [55]	7-544 (37-236)	444 (283) [501]	157-948 (205-547)	.02[b]
Age at first use, y	19.8 (4.0)	16-31	18.7 (1.3)	17-21	.37
Frequency of use in past 3 mo, d/mo	0.6 (0.9)	0-3.3	0.0	0-0	.02
GHB					
No. of subjects who have ever used	9/14		7/10		
Time since last use, d	221 (311) [117]	23-1010 (30-205)	549 (289) [499]	98-1012 (424-694)	.03[b]
Age at first use, y	22.0 (4.2)	16-31	20.0 (1.2)	18-21	.17
Frequency of use in past 3 mo, d/mo	0.2 (0.3)	0-0.7	0.0 (0.0)	0-0	.10
Ketamine					
No. of subjects who have ever used	8/14		6/10		
Time since last use, d	321 (282)	29-860	324 (370)	33-1012	.99
Age at first use, y	21.5 (2.5)	18-25	21.3 (0.8)	20-22	.86
Frequency of use in past 3 mo, d/mo	0.5 (1.3)	0-3.7	0.2 (0.3)	0-0.7	.57

Abbreviations: GHB, γ-hydroxybutyrate; HPU, hallucinogen-preferring user; IQR, interquartile range; MDMA, 3,4-methylenedioxymethamphetamine; MPU, MDMA-preferring user.

[a] Compares the MPU and HPU groups. Determined by unpaired, 2-tailed *t* test unless otherwise indicated.

[b] Determined by Mann-Whitney nonparametric signed rank test owing to absence of normal distribution.

Drug users were divided into 2 groups according to lifetime exposures to MDMA and hallucinogens: MDMA-preferring users (MPUs) (n=14) and hallucinogen-preferring users (HPUs) (n=10) with an MDMA/hallucinogen number of lifetime exposures ratio of greater than and less than 1.0, respectively.

DRUG ANALYSIS IN URINE AND HAIR

For the 7 days before scanning, abstinence from illegal drug intake was confirmed by urine screen results every 2 to 3 days, using a standard, 9-panel drug screen kit (Syva RapidTest d.a.u.9; Syva Company, Dade Behring Inc, San Jose, California). Self-reported recent use of MDMA was confirmed by gas chromatography mass spectroscopy analysis[42] of 3.5-cm scalp hair segments covering approximately 3 months before the scan. The measurements of MDMA in the hair were used primarily to confirm reported drug use, mostly because they represent an average concentration of a 3-month period and because factors such as hair color and hair treatments can affect the absolute measurements.

PET IMAGING

All participants underwent PET scanning with both carbon 11 (^{11}C)–labeled 3-amino-4-[2-[(di(methyl)amino)methyl] phenyl] sulfanylbenzonitritile (DASB) and fluorine 18 (^{18}F)–labeled altanserin on an 18-ring scanner (GE-Advance scanner; GE, Milwaukee, Wisconsin) operating in 3-dimensional

acquisition mode and producing 35 image sections with an intersection distance of 4.25 mm. The total axial field of view was 15.2 cm with an approximate in-plane resolution of down to 5 mm. All participants were scanned in a resting state.

For [^{18}F]altanserin, subjects underwent a 40-minute scan under tracer steady-state conditions, as described by Pinborg et al[43]; for [^{11}C]DASB, a dynamic 90-minute emission recording was initiated after intravenous injection of the radiotracer. The PET scans were performed between November 23, 2005, and December 17, 2007. For 16 drug users and 14 controls, the 2 PET scans were performed on the same day; for the remaining participants, there was a median gap between the 2 PET scans of 5 days (range, 1-220 days). Because of a technical failure in the analysis procedure of [^{18}F]altanserin blood metabolite samples, the binding outcome measure from 3 drug users and 1 control could not be quantified. Thus, data for the analysis of serotonin$_{2A}$ receptor binding were available from 21 users (3 women and 18 men, mean [SD] age, 24.6 [4.1]; range, 20.1-34.7 years) and 20 controls (3 women and 20 men, 23.7 [3.4]; range, 19.6-32.6 years).

The outcome parameter for [^{18}F]altanserin binding was the binding potential of specific tracer binding (BP_P). The cerebellum was used as a reference region because it represents nonspecific binding.[43] In the steady state, BP_P is defined as:

$$BP_P = [(C_{VOI} - C_{ND})/C_P] = f_p(B_{max}/K_d),$$

where C_{VOI} and C_{ND} are the steady-state mean count density in the volume of interest (VOI) and the reference region, respectively, C_P is the steady-state activity of nonmetabolized tracer in plasma, f_p is the free fraction of radiotracer, B_{max} is the density of receptor sites available for tracer binding, and K_d is the dissociation constant reflecting affinity of the radiotracer to the receptor. The outcome parameter of the [^{11}C]DASB binding is the nondisplaceable binding potential (BP_{ND}). We used a modified reference tissue model designed for quantification of [^{11}C]DASB (Multilinear Reference Tissue Models [MRTM/MRTM2]), as described and evaluated by Ichise et al[44] (PMOD version 2.9; PMOD Technologies, Zurich, Switzerland). Further details on [^{18}F]altanserin[45] and [^{11}C]DASB[46] imaging and quantification are available.

MAGNETIC RESONANCE IMAGING

Magnetic resonance (MR) imaging of the brain was conducted (Siemens Magnetom Trio 3T MR; Siemens Medical Solutions, Malvern, Pennsylvania; with an 8-channel head coil; Invivo Corporation, Gainesville, Florida). High-resolution, 3-dimensional, T1-weighted, sagittal, magnetization-prepared, rapid gradient-echo scans of the head and T2-weighted scans of the whole brain were performed. Both T1 and T2 images were corrected for spatial distortions due to nonlinearity in the gradient system of the scanner[47] (Gradient Nonlinearity Distortion Correction software, distributed by the Biomedical Informatics Research Network; http://www.nbirn.net). Subsequently, nonuniformity correction of the T1 images was performed with 2 iterations of the N3 program.[48] The resulting T1 images were intensity normalized to a mean value of 1000. To enable extraction of the PET signal originating from gray matter voxels, MR images were segmented into gray matter, white matter, and cerebrospinal fluid tissue classes using SPM2 (Wellcome Department of Cognitive Neurology, University College London, London, England) and the Hidden Markov random field model as implemented in the SPM2 VBM (voxel-based morphometry) toolbox (http://dbm.neuro.uni-jena.de/vbm/). This was done for all VOIs except the midbrain, where segmentation is considered unreliable; therefore, all midbrain voxels were included in the analysis. A brain mask based on the gradient nonlinearity–corrected T2 image was used to exclude extracerebral tissue.

Table 2. Hallucinogens Used by the Participants

Name (Full Name) of Hallucinogen	No. of Participants Who Have Ever Used the Substance
Psilocybin (*O*-phosphoryl-4-hydroxy-*N,N*-dimethyltryptamine)	19
LSD (lysergic acid diethylamide)	18
2-CB (2,5-dimethoxy-4-bromophenethylamine)	15
2-CI (2,5-dimethoxy-4-iodophenethylamine)	11
DMT (dimethyltryptamine)	11
Salvia divinorum	8
2-CE (2,5-dimethoxy-4-ethylphenethylamine)	5
LSA (D-lysergamide; ergine)	5
Mescaline (3,4,5-trimethoxyphenethylamine)	4
DOB (2,5-dimethoxy-4-bromoamphetamine)	3
DIPT (diisopropyltryptamine)	3
AMT (α-methyltryptamine)	3
TMA-2 (1-[2,4,5-trimethoxyphenyl]propan-2-amine, 2,4,5-trimethoxyamphetamine)	3
Ayahuasca	3
2-CT-4 (2,5-dimethoxy-4-isopropylthiophenethylamine)	2
2-CT-7 (2,5-dimethoxy-4-n-propylthiophenethylamine)	2

VOLUMES OF INTEREST

The VOIs were automatically delineated on each participant's transaxial MR image sections in a user-independent fashion.[49] With this approach, a template set of 10 MR images is automatically coregistered to a new participant's MR image. The identified transformation parameters are used to define VOIs in the new person's MR image space and, through coregistration, these VOIs are transferred onto the PET images.

For both the SERT and serotonin$_{2A}$ receptor, we computed an average binding potential for neocortex for each participant, and this served as the primary outcome. This region consisted of a volume-weighted average of 8 cortical regions (orbitofrontal cortex, medial inferior frontal cortex, superior frontal cortex, superior temporal cortex, medial inferior temporal cortex, sensory motor cortex, parietal cortex, and occipital cortex). In addition, for SERT binding, the pallidostriatum, midbrain, amygdala, and thalamus were defined. This was done to limit the number of statistical comparisons; the approach is justified because there is a high correlation between binding in high-binding regions.[50] The cerebellum (except the vermis) was defined and used for nonspecific binding measurements for both markers, since this region has only negligible numbers of serotonin$_{2A}$ receptors and SERT.[43,51-53] Within a high-binding subcortical region (volume-weighted average of pallidostriatum and thalamus) and neocortex, the ratio between gray matter volume and the sum of the white plus gray matter volumes was computed.

STATISTICAL ANALYSIS

For evaluation of agonistic effects on cerebral serotonin$_{2A}$ receptor binding by MDMA and hallucinogens, serotonin$_{2A}$ agonist users (n=21) were compared with the controls using a 2-tailed nonparametric test (Mann-Whitney).

Group differences between MPUs, HPUs, and controls in binding of the 2 serotonergic markers, sociodemographic data, tobacco and alcohol use, and scanning-related variables were tested using 1-way analyses of variance with subsequent correction for multiple comparisons by the Tukey test. In cases in

which model assumptions were violated, a nonparametric signed rank test was used (Kruskal-Wallis test with Dunn multiple comparison test). Drug use was compared between MPUs and HPUs using an unpaired 2-tailed *t* test (Table 1); if model assumptions were violated, a Mann-Whitney nonparametric signed rank test was used.

Because cerebral SERT binding shows season-dependent changes[54,55] and both cerebral SERT and serotonin$_{2A}$ receptor binding show an age-dependent decline[45,56-59] as well as a relation to body mass index,[45] the following factors were tested, one at a time, as covariates in a multiple regression analysis, with regional binding potential as the response variable and the 3 subject groups as additional predictor variables: age, body mass index, and daylight minutes on the day of the [^{11}C]DASB scan. Furthermore, because of the potential confounding effects of the significant group difference in the education score and use of amphetamine, cocaine, and cannabis in the 3 months before the PET scan, these factors were tested in this model. None of these potential confounders were statistically significant, and the effect sizes and 95% confidence intervals (CIs) of the subject group variable showed only minor changes in the different models. Accordingly, these variables were excluded from the final models.

To explore whether there were dose-response effects on the serotonergic brain markers of accumulated use of MDMA and/or hallucinogens, the following factors were tested separately within the total group of drug users as the independent variable in a linear regression analysis, with regional SERT or serotonin$_{2A}$ receptor binding as the dependent variable and both with and without age as a covariate: (1) the logarithmic lifetime number of consumed MDMA tablets (23 participants for SERT binding and 20 participants for serotonin$_{2A}$ receptor binding) and (2) the logarithmic number of lifetime exposures to hallucinogens (21 participants for SERT binding and 19 for serotonin$_{2A}$ receptor binding). Model assessment was performed via residual-based techniques[60] showing that the functional relationship was adequately described by the logarithmic transform of the predictor. For the serotonin$_{2A}$ receptor binding, the number of lifetime exposures to either of the 2 drugs (lifetime serotonin$_{2A}$ agonist use) was also tested as a dependent variable in this model.

In addition, we fitted a log-logistic 4-parameter model allowing us to effectively use all observations to quantify the dose-response relationship.[61] At the cost of a small increase in model complexity, this model gives more precise predictions of the response variable at high (or low) doses. The effect can, in this setup, naturally be quantified by the dose required to reduce the average dose-response curve halfway from the value at dose 0 to the dose at infinity.

To further test whether the presynaptic and postsynaptic serotonergic markers showed any signs of recovery over time, the number of days since last drug use before the scan of either MDMA alone, hallucinogens alone, and, for the [^{18}F]altanserin binding, also any of the 2 drug types together (time since last serotonin$_{2A}$ agonist use) were tested as dependent variables in the same model. However, to test whether recovery of SERT binding was influenced by the extent of MDMA use, adjustment for the lifetime number of ingested MDMA tablets was added to the model. For the analysis of serotonin$_{2A}$ receptor binding, the drug users were divided into a group with recent use of a serotonin$_{2A}$ agonist (a maximum of 1 month since the last use [n = 10]) and a group with the last use more than 1 month before the scan (n = 11), and regional serotonin$_{2A}$ receptor binding was compared between these 2 groups using the unpaired *t* test. Finally, we tested whether our previous detection of an inverted U–shaped relationship between cortical serotonin$_{2A}$ receptor binding and the subcortical SERT binding[62] could be replicated in the present data set. The statistical approach for this analysis was the same as an approach described earlier.[62]

RESULTS

SOCIODEMOGRAPHIC DATA AND SCANNING PARAMETERS

Table 3 lists demographic variables, parent socioeconomic level, and alcohol and tobacco use variables; **Table 4** indicates some of the scanning-related variables. No significant group differences were observed for age, body mass index, parent socioeconomic level, injected dose or specific activity of [^{11}C]DASB and [^{18}F]altanserin, number of daylight minutes on the day of the [^{11}C]DASB scan, and cortical and subcortical gray matter ratio. Educational level differed significantly between the groups, with MPUs being less educated than participants in the other 2 groups.

There was no significant difference in alcohol use between the 3 groups, and the use of cannabis did not differ significantly between MPUs and HPUs. In contrast, tobacco smoking differed between the 3 groups; the HPUs started smoking later than did the individuals in the 2 other groups, and the MPUs had smoked more cigarettes during their entire lives (as estimated by the number of pack-years) and during the week before the scan compared with the 2 other groups.

Details about the reported use of MDMA, hallucinogens, cannabis, amphetamines, cocaine, GHB (γ-hydroxybutyrate), and ketamines are given in Table 1. None of the participants reported use of MDMA or hallucinogens for a minimum of 11 and 14 days, respectively, before any of the brain scans. Negative results of urine tests confirmed abstinence from MDMA. Measurement of MDMA content in scalp hair segments corresponding to use of the drug approximately 3 months before the PET scan confirmed self-reported MDMA use in 13 of 14 participants (median, 1.8 ng of MDMA per milligram of hair; range, 0.2-11.2 ng/mg). No MDMA was detected in the hair sample from a woman who reported use of MDMA, 90 mg, 36 days before the PET scan. Excluding this individual from the analysis had little effect on the results, although the group difference in SERT binding in the thalamus was slightly larger ($P = .04$) when she was excluded. The results presented in the next sections were obtained including the data on this woman.

SERT BINDING

There were statistically significant between-group differences in SERT binding in the pallidostriatum ($F = 7.89$; $P = .001$), neocortex ($F = 23.05$; $P < .001$), and amygdala ($F = 16.58$; $P < .001$). In all 3 regions, MPUs had lower SERT BP_{ND} than did HPUs (difference in the striatum, 0.33 BP_{ND} units (U) [95% CI, 0.09-0.57]; neocortex, 0.10 BP_{ND} U [0.03-0.16]; and amygdala, 0.40 BP_{ND} U [0.14-0.65]) and controls (difference in the striatum, 0.30 BP_{ND} U [0.09-0.50]; neocortex, 0.15 BP_{ND} U [0.09-0.20]; and amygdala, 0.49 BP_{ND} U [0.28-0.71]). Further results are shown in **Figure 1** and **Figure 2**. In comparison with controls, the regional SERT binding in MPUs was lower by 19% in the pallidostriatum, 32% in the amygdala, and 56% in the neocortex (40% in the orbitofrontal cortex,

Table 3. Characteristics of the Study Groups

Characteristic	Group, Mean (SD) [Range]			*P* Value[a]	Significance		
	MPU (n=14)	HPU (n=10)	Control (n=21)		MPU vs Control	HPU vs Control	MPU vs HPU
Age, y	25.5 (4.8) [20.1-34.7]	23.3 (2.25) [20.1-27.9]	23.8 (3.4) [19.6-32.6]	NS	NS	NS	NS
Sex							
Male	12	9	17				
Female	2	1	4	NA	NA	NA	NA
BMI	23.8 (2.2) [21.1-29.2]	24.0 (3.4) [19.5-30.3]	23.5 (3.1) [19.3-28.5]	NS	NS	NS	NS
Educational level[b]	2.4 (1.7) [1-5]	3.9 (1.7) [1-5]	3.8 (1.6) [1-5]	.04[c]	NS	NS	NS
Parent socioeconomic level	2.9 (0.9) [1.5-4.0]	3.0 (1.1) [1.0-4.0]	3.4 (0.6) [2.0-4.0]	NS	NS	NS	NS
Alcohol							
Time since last use, d	7.6 (7.5) [2-26]	8.6 (7.8) [3-23]	8.0 (14.0) [2-52]	NS	NS	NS	NS
Age at first use, y	12.9 (1.4) [11-16]	14.5 (1.4) [11-16]	13.7 (1.3) [10-16]	NS	NS	NS	NS
Length of use, mo	150 (59) [82-271]	105 (31) [60-154]	125 (44) [59-270]	NS	NS	NS	NS
Frequency of use, d/mo	4.5 (2.7) [0.6-9.2]	3.7 (2.3) [0.2-6.1]	4.2 (3.2) [0.5-12.9]	NS	NS	NS	NS
Amount consumed in past 3 mo, mean, U/wk[d]	8.8 (6.0) [1-20]	9.2 (6.8) [0.3-20.6]	9.5 (6.3) [0-21]	NS	NS	NS	NS
Tobacco							
No. of current smokers	9/14	5/10	4/21				
Age at first use, y	13.1 (2.8) [8-18]	16.9 (2.5) [14-23]	14.1 (2.2) [12-17]	.01[c]	NS	NS	[c]
Amount smoked during week before scan, No./d	7.8 (10.7) [0-35]	5.1 (6.7) [0-20]	2.6 (6.4) [0-25]	.03[c,e]	[c]	NS	NS
Pack-years, No.[f]	4.3 (5.5) [0-20]	1.0 (1.9) [0-6]	1.0 (2.1) [0-8]	.02[c]	[c]	NS	NS

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HPU, hallucinogen-preferring user; MPU, 3,4-methylenedioxymethamphetamine–preferring user; NA, not applicable; NS, not significant.
[a] Determined by equivalence test unless otherwise indicated.
[b] Indicated by a score of 1 to 5 that reflects the level of vocational training or degree.
[c] *P* value <.05.
[d] One unit of alcohol is defined as the alcohol content of a 330-mL beer of regular strength (Danish beer [3.7%], approximately 12 g of alcohol).
[e] Determined by Kruskal-Wallis nonparametric signed rank test with Dunn multiple comparisons test.
[f] Indicates the calculated number of years of smoking 20 cigarettes per day.

Table 4. Scan-Related Variables

	Group, Mean (SD) [Range]		
	MPU (n=14)	HPU (n=10)	Control (n=21)
[^{11}C]DASB			
Injected dose, MBq/kg of body weight	6.5 (1.1) [4.3-8.3]	6.9 (1.2) [5.2-9.4]	6.5 (1.3) [4.6-8.9]
Specific activity, GBq/μmol	34.0 (12.0) [16-57]	30.5 (12.9) [18-53]	27.4 (15.1) [10-56]
Daylight on day of scan, min	608 (200) [422-1052]	748 (219) [439-978]	743 (284) [422-1052]
[^{18}F]altanserin[a]			
Injected dose, MBq/kg of body weight	3.7 (0.1) [3.6-4.0]	3.6 (0.3) [2.9-3.8]	3.7 (0.1) [3.2-3.7]
Specific activity, GBq/μmol	52.7 (21.1) [21-84]	54.5 (27.8) [16-98]	52.1 (31.7) [11-126]
Cortical gray matter ratio	0.69 (0.02) [0.65-0.72]	0.70 (0.02) [0.67-0.72]	0.70 (0.02) [0.65-0.74]
Subcortical gray matter ratio	0.70 (0.04) [0.63-0.76]	0.69 (0.03) [0.62-0.75]	0.71 (0.04) [0.64-0.77]

Abbreviations: [^{11}C]DASB, carbon 11 (^{11}C)–labeled 3-amino-4-[2-[(di(methyl)amino)methyl]phenyl]sulfanylbenzonitrile; [^{18}F]altanserin, fluorine 18 (^{18}F)–labeled altanserin; HPU, hallucinogen-preferring user; MPU, 3,4-methylenedioxymethamphetamine–preferring user.
[a] For the [^{18}F]altanserin data: 12 MPUs, 9 HPUs, and 20 controls (see the "PET Imaging" subsection of the "Methods" section).

53% in the medial inferior frontal cortex, 61% in the superior frontal cortex, 48% in the superior temporal cortex, 51% in the medial inferior temporal cortex, 66% in the sensory motor cortex, 47% in the parietal cortex, and 73% in the occipital cortex). The same pattern was detected in the thalamus, although the group difference had only borderline significance ($F=2.6$, $P=.08$), and no group effect was seen in the midbrain. Inclusion of potential confounding factors (key demographic parameters and use of drugs other than MDMA/hallucinogens) in the model did not change these results.

There was a significant negative correlation between the logarithmic accumulated lifetime intake of MDMA tablets and SERT BP_{ND} in all investigated brain regions (ex-

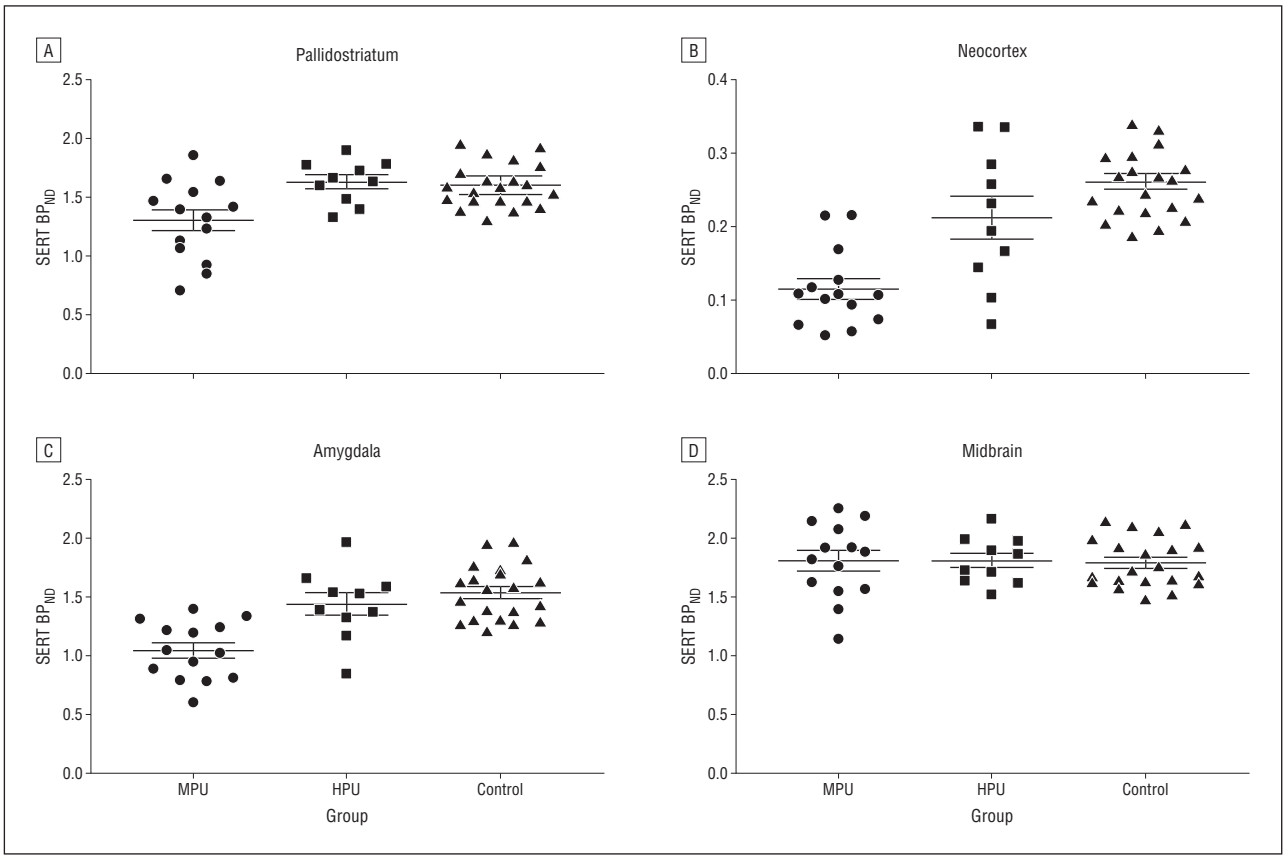

Figure 1. Binding potentials of carbon 11 (^{11}C)–labeled 3-amino-4-[2-[(di(methyl)amino)methyl]phenyl]sulfanylbenzonitrile in the pallidostriatum (A), neocortex (B), amygdala (C), and midbrain (D) in the 3,4-methylenedioxymethamphetamine (MDMA)–preferring users (MPU), hallucinogen-preferring users (HPU), and control participants (control) groups. BP_{ND} indicates nondisplaceable binding potential; SERT, serotonin transporter. The longer horizontal lines represent mean; the shorter horizontal lines, standard deviations.

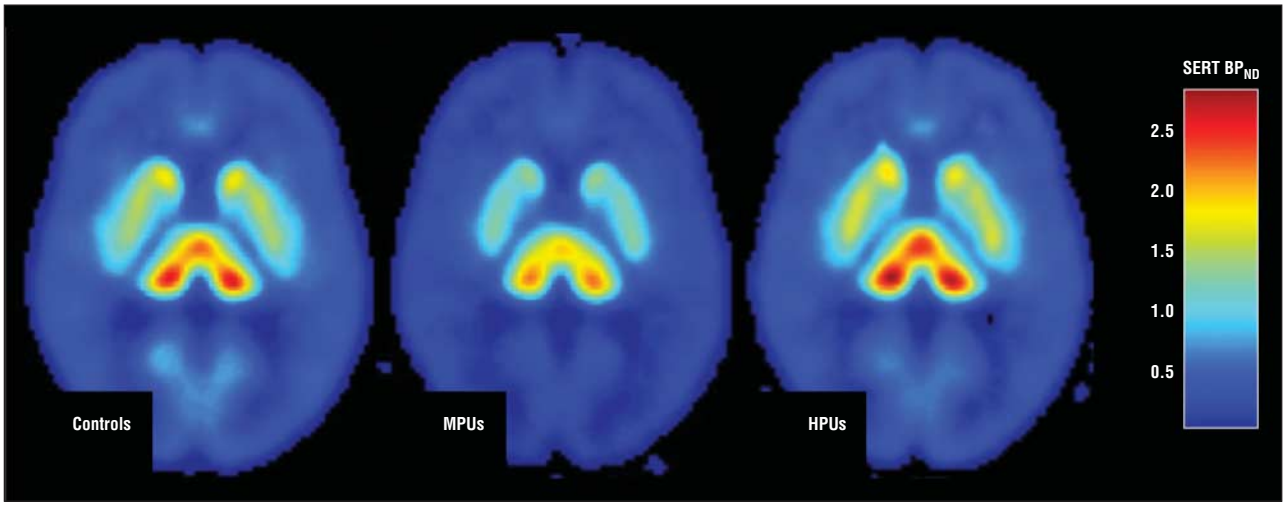

Figure 2. Parametric average SERT BP_{ND} images of the MPUs, HPUs, and control groups. Abbreviations are defined in the legend to Figure 1.

cept for the midbrain). Thus, a doubling in the consumption of tablets corresponded to a decrease in SERT BP_{ND} of 0.021 in the neocortex (95% CI, –0.029 to –0.014; $P<.001$), 0.062 in the pallidostriatum (–0.103 to –0.021; $P=.005$), 0.067 in the thalamus (–0.113 to 0.022; $P=.006$), and 0.075 in the amygdala (–0.106 to 0.045; $P<.001$) (**Figure 3**).

The $\log_2$ to the number of days since the last use of MDMA was positively related to SERT BP_{ND} in the pallidostriatum (a BP_{ND} increase of 0.14 per doubling of days since the last use of MDMA [95% CI, 0.05-0.23]; $P=.004$), the amygdala (0.12 BP_{ND} per doubling of days [0.04-0.21]; $P=.008$), and the thalamus (0.11 BP_{ND} per doubling of days [0.002-0.22]; $P=.046$). No such relationship was detected in the neocortex (estimate, 0.005 BP_{ND} [–0.021 to 0.032]; $P=.67$).

Both the regional dose-response relationship and the recovery of SERT binding remained statistically signifi-

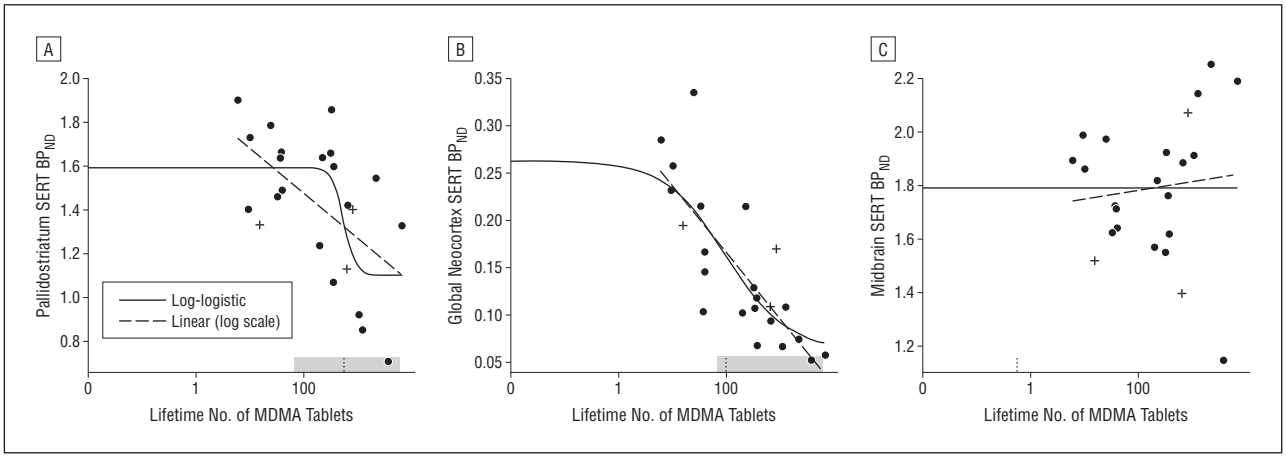

Figure 3. Estimated log-logistic curve and estimated linear relationship between the log number of lifetime usage and SERT BP_{ND} in the pallidostriatum (A), global neocortex (B), and midbrain (C). For the log-logistic curve, the estimated dose required to reduce the average dose-response curve halfway from the value at dose 0 to the dose at infinity (ED_{50}) with 95% bootstrap percentile confidence bands (R=5000) (shaded sections) are marked on the x-axis for A and B. Thalamus and amygdala are not shown, but there were also significant dose-response relationships in these 2 regions, as presented in the "Results" section. Other abbreviations are defined in the legend to Figure 1.

cant in the extended model in which both parameters (the lifetime number of ingested MDMA tablets and the number of days since last MDMA use) were included. The only exception was the thalamus, with the relationship between the number of days since last use and SERT BP_{ND} no longer being significant in this model (regional estimates with 95% CIs of BP_{ND} increases per doubling of days since the last use of MDMA with adjustment for the lifetime number of ingested MDMA tablets: pallidostriatum: 0.11 [0.02-0.19; P=.02], amygdala: 0.07 [0.01-0.14; P=.02], midbrain: 0.10 [0.01-0.19; P=.02], thalamus: 0.07 [–0.03 to 0.18; P=.17], and neocortex: –0.01 [–0.03 to 0.01; P=.18]). For the pallidostriatum, the positive correlation between the number of days of MDMA abstinence and SERT BP_{ND} with adjustment for the lifetime number of ingested MDMA tablets is illustrated in **Figure 4**, in which the dotted line marks the estimated number of days of abstinence from MDMA required for "normalization" of SERT binding (to reach the average of BP_{ND} in the control group level). For the use of hallucinogens, neither a dose-response relationship nor signs of recovery of SERT binding was observed. There were no significant relationships between SERT binding and the accumulated lifetime use of hallucinogens or the time since last use of hallucinogens (neither linear nor logarithmic).

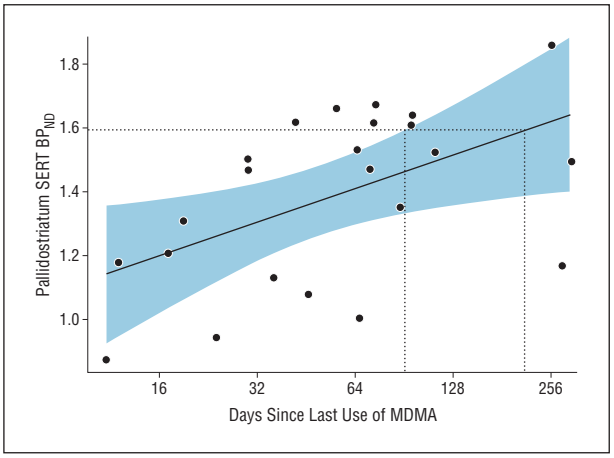

Figure 4. Partial residual plot (adjusted to the median number of lifetime use of 3,4-methylenedioxymethamphetamine [MDMA], 314 tablets). The horizontal dashed line is the estimated SERT BP_{ND} at dose 0 from the log-logistic model (Figure 3A). For participants with a median lifetime use, we see an expected return to the normal SERT level occurring after an average of 212 days (right vertical dashed line). Based on the lower 95% confidence limit (top edge of the shaded area), SERT BP_{ND} will reach the average level among controls after 91 days (left vertical dashed line), whereas the upper limit estimate is beyond the human lifespan. Other abbreviations are defined in the legend to Figure 1.

SEROTONIN$_{2A}$ RECEPTOR BINDING

When serotonin$_{2A}$ agonist users (MPUs and HPUs) were compared with controls, serotonin$_{2A}$ agonist users had slightly lower neocortical serotonin$_{2A}$ BP_{ND} (median, 1.51; range, 1.16-2.09 BP_{ND} U) than controls (median, 1.66; range, 1.31-3.30 BP_{ND} U) (2-tailed Mann-Whitney test, P=.03) (**Figure 5**A and **Figure 6**). The regional serotonin$_{2A}$ receptor BP_P in serotonin$_{2A}$ agonist users in comparison with controls was decreased by 9% in the neocortex (13% in the orbitofrontal cortex, 10% in the medial inferior frontal cortex, 7% in the superior frontal cortex, 11% in the superior temporal cortex, 13% in the medial inferior temporal cortex, 7% in the sensory motor cortex, 8% in the parietal cortex, and 4% in the occipital cortex). Two control participants had very high serotonin$_{2A}$ BP_P values (easily identified on Figure 5A); these 2 values were carefully scrutinized, and there appeared to be no justification for removing them from the sample. However, without these 2 participants, the difference between serotonin$_{2A}$ agonist users and controls was no longer statistically significant (2-tailed Mann-Whitney test, P=.10).

When MPUs and HPUs were analyzed in separate groups, no significant group difference in global neocortical serotonin$_{2A}$ receptor binding between MPUs, HPUs, and controls could be demonstrated (Kruskal-Wallis test, P=.07). Analysis of the potential effect on serotonin$_{2A}$ receptor binding of (1) the accumulated lifetime use of either MDMA or hallucinogens or of any of the 2 drug types or (2) the time since the last use of MDMA, hallucinogen, or any of these 2 drug types as either continuous or cat-

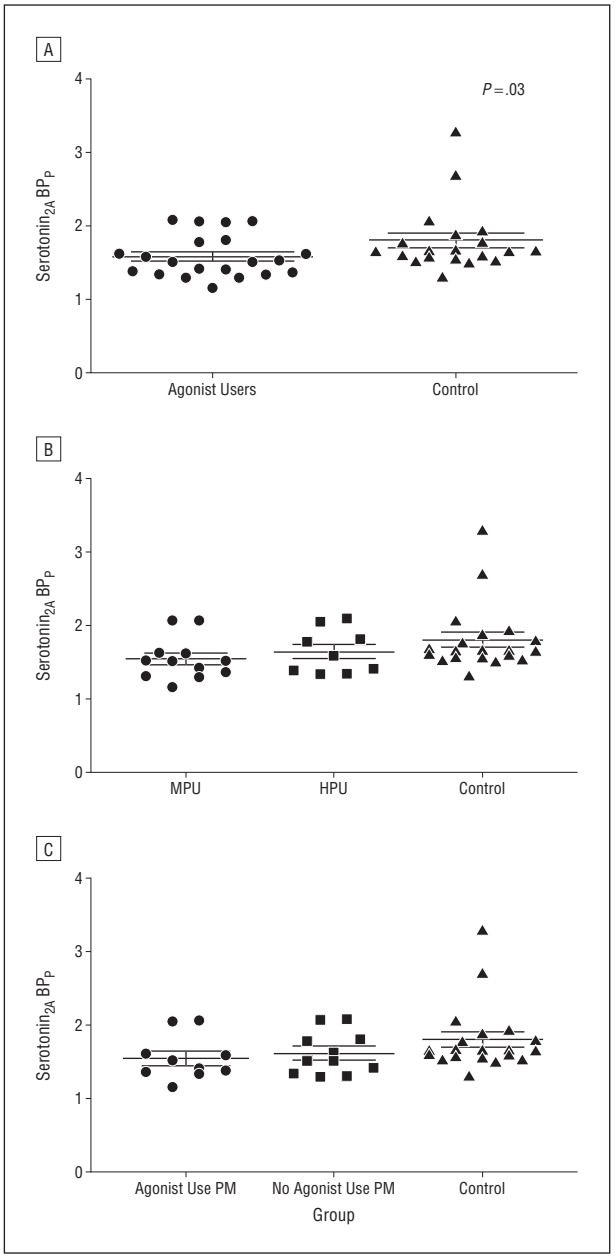

Figure 5. Group comparisons of neocortical serotonin$_{2A}$ receptor binding. Agonist users consist of both 3,4-methylenedioxymethampetamine–preferring users (MPUs) and hallucinogen-preferring users (HPUs). BP_P indicates binding potential of specific tracer binding; HPU, hallucinogen-preferring user; PM, during the past month; and MPU, MDMA-preferring user. The longer horizontal lines represent mean; the shorter horizontal lines, standard deviations.

egorical variables (Figure 5C) did not reveal any significant relationships. Neither logarithmic transformation of the data nor inclusion of potential confounding factors in the model (age, body mass index, daylight minutes, and the use of drugs other than MDMA/hallucinogens) changed these results.

SEROTONIN$_{2A}$ RECEPTOR VS SERT BINDING

In the group of drug users, a significant inverted U–shaped relationship was detected between neocortical serotonin$_{2A}$ receptor binding and pallidostriatal SERT binding (*P* value for second-order term, .046).

COMMENT

To our knowledge, this is the first study in which relevant presynaptic and postsynaptic markers were assessed simultaneously within the same MDMA-using individuals and also the first study to image in vivo cerebral serotonergic markers in recreational users of hallucinogens. We found that MDMA-preferring users, but not hallucinogen-preferring users, had profound reductions in cerebral SERT binding. However, the cortical serotonin$_{2A}$ receptor binding was only slightly decreased among the serotonin$_{2A}$ agonist users compared with non–drug-using controls.

SERT BINDING AND USE OF MDMA

We found that use of MDMA on at least 12 separate occasions was associated with reduced SERT binding in the pallidostriatum, amygdala, and neocortex, but not in the midbrain, and was associated with only borderline decreased SERT binding in the thalamus. We also identified a negative correlation between the number of reported lifetime MDMA exposures and SERT binding in all investigated brain regions except the midbrain, which suggests a dose-response relationship between the extent of MDMA use and SERT reduction; a doubling in the number of ingested lifetime MDMA tablets leads to a pallidostriatal decrease in SERT binding of 0.6 BP_{ND} U.

These findings concur well with earlier studies in animals (see Capela et al[4] for review) as well as in humans, in which decreased SERT binding has been observed in moderate to heavy,[17-21] but not in light, MDMA users.[63,64] As in the present study, a negative association between SERT binding and the extent of MDMA use has been detected in most of these earlier imaging studies in humans.[17,19,21,65-67]

Also in line with our data, several studies support reversibility of the SERT binding changes in relation to MDMA use; cerebral SERT binding is reduced in MDMA users with a relatively short abstinence of 24 days,[18] 70 days (women only),[17] and 145 days,[19] but is normal in MDMA users with longer abstinence periods of 514,[18] 885,[17] and 1000[68] days. A follow-up study by Buchert et al[69] of their previous cohort,[18] as well as 2 independent studies,[19,66] further support the notion that a long-term recovery of SERT availability takes place after termination of MDMA use. By extrapolation, we estimate that full recovery of pallidostriatal SERT binding takes place approximately 200 days after the last MDMA dose; this estimate is in accordance with Buchert et al,[69] who suggest that full recovery takes from several months to a few years. As a consequence of our study design, this calculation is based on interindividual rather than intraindividual data.

A number of studies in rats[9,10,16] and nonhuman primates[14,15] have pointed to a regionally dependent recovery of SERT binding following MDMA abstinence. Thus, in neocortical areas, the reduction in SERT is more protracted[13,15] or maybe even permanent[14] when compared with regions closer to the raphe nuclei, and some studies[11,12] suggest that the recovery of serotonergic neurons

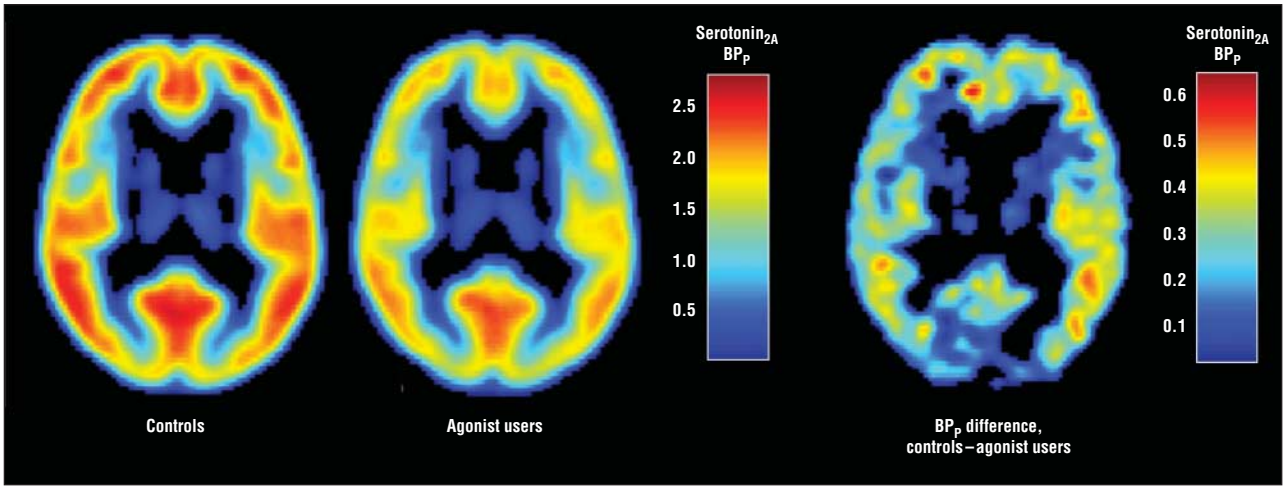

Figure 6. Parametric average serotonin$_{2A}$ binding potential of specific tracer binding (BP_P) images of control participants and agonist users and the difference between the 2 groups.

depends on the distance from raphe, with the more distant areas, such as the occipital cortex, showing the least-complete recovery. Our data are in agreement with this hypothesis because the most pronounced decrease in SERT binding among MDMA users was detected in the cortex (an average of 56% in the global neocortex, ranging from 40% in the orbitofrontal cortex to 73% in the occipital cortex). In addition, no correlation with length of MDMA abstinence was present in the cortex, possibly because this region was the least recovered. It cannot be excluded that a lower and more noisy PET signal caused by lower density of SERT could affect the ability to detect a significant relationship in the cortex, although the reliability of BP_{ND} is relatively good (intraclass correlation coefficients: temporal cortex, 0.82; occipital cortex, 0.85; and frontal cortex, 0.55).[70] As further support of this observation of a particular cortical vulnerability to MDMA, decreased SERT binding was exclusively detected in cortical areas, and most pronounced in the occipital cortex, in a recent PET study using [^{11}C]DASB in a group of low to moderate MDMA users.

We did not identify any significant between-group differences in SERT binding in the midbrain, consistent with ex vivo studies using different techniques[9,10,14,16,22,71-73] in which the serotonergic cell bodies in raphe nuclei were found to be unaffected by MDMA treatment. In contrast, some of the early in vivo imaging studies[18,65,66] reported decreased midbrain SERT binding in MDMA users and in female but not male users.[17] However, the radioligands used in the early neuroimaging studies of MDMA users did not allow for the assessment of cortical binding. In the 2 other [^{11}C]DASB PET studies of current MDMA users published so far,[19,21] midbrain SERT binding was unaltered, and the regionally most pronounced decline in SERT binding was, as in our study, seen in the occipital cortex of MDMA users: 46% in the study by McCann et al[19] and 54% in the study by Kish et al.[21] We have no ready explanation for why some of the early studies found signs of decreased midbrain SERT binding and the 2 latest studies did not. Differences in the radioligand and quantification of MDMA or in the duration and extent of MDMA use could play a role.

It is not entirely clear whether a temporary decrease in cerebral SERT binding in human users of MDMA (as suggested by our results as well as by the results of related studies[18,19,69]) should be viewed as an adaptive neural mechanism or whether it reflects toxic exposure of serotonergic neurons with a secondary reinnervation pattern of serotonergic axons. Our data do not allow a distinction between these possible mechanisms, but the issue is briefly considered here. In in vitro models, formation of toxic MDMA metabolites,[4] dopamine-induced oxidative stress in serotonin terminals,[74] and serotonin$_{2A}$ and dopamine D_1 receptor–mediated hyperthermia[31] are all factors involved in MDMA neurotoxicity. The MDMA-related changes in SERT binding in animals and humans have often been interpreted as being the consequence of a loss of serotonergic axons and terminals with subsequent formation of new irregular fibers.[4,13,75] Interestingly, one of the prevailing arguments for MDMA toxicity is the presence of a relative cerebral serotonin depletion, low serotonin uptake, and/or low SERT binding in MDMA-treated animals (see Green et al[75] and Capela et al[4] for review). As recently discussed by Baumann et al,[76] it is questionable whether profound serotonin depletion is equivalent to MDMA neurotoxicity against serotonin neurons. Reductions in cerebral serotonin markers can occur in the absence of neuronal damage.[71,72,77,78] Moreover, Wang et al[79] found that, in contrast to serotonin depletion by the serotonergic neurotoxin 5,7-dihydroxytryptamine, MDMA-induced serotonin depletion of about 50% in cortical and subcortical brain regions was not accompanied by changes in the levels of glial fibrillary acidic protein, a sensitive marker of neuronal damage. Some[35,36] (but not all[37]) studies report decreased SERT binding after nonneurotoxic, pharmacologically induced, chronic serotonin depletion in rats, suggesting that low serotonin levels may be associated with a downregulation of SERT.

In summary, from the present data and published imaging studies,[17-21,63-67] we conclude that heavy (but not light) use of MDMA is associated with the decreased availability of cerebral SERT binding in humans. Because it is not fully understood whether the observed normal-

ization of SERT binding represents axonal toxic effects with subsequent new formations of serotonergic projections or rather a temporary downregulation of SERT, we prefer not to use the term *neurotoxicity* in the context of our data. Correlating the changes of the serotonergic markers with functional (such as psychiatric symptoms and neuropsychological tests) and structural (such as MR morphometry and diffusion tensor imaging) data could add to the interpretation of the functional significance of such findings.

SERT BINDING AND USE OF HALLUCINOGENS

Hallucinogen users had no signs of serotonergic impairment; instead, they had normal cerebral SERT binding. Although the primary effect of hallucinogens is through their actions as serotonin$_{2A}$ receptor agonists, some degree of SERT reduction could have been expected a priori. Cortical serotonin$_{2A}$ receptor stimulation exerts a negative feedback effect on raphe serotonin neurons[34]; in addition, hallucinogens such as LSD and psilocybin inhibit raphe serotonin neurons via serotonin$_{1A}$ autoreceptor activation.[80,81] Furthermore, recent in vitro studies[82,83] have suggested that some hallucinogens, in particular some of the tryptamines, are substrates of the SERT. Taken together, these findings suggest that the use of hallucinogens could lead to downregulation of SERT, but, in fact, hallucinogen treatment of animals does not cause a depletion of cerebral serotonin.[28]

Because the common mechanism of action of hallucinogenic drugs is their stimulation of serotonin$_{2A}$ receptors[84] and because these drugs, in contrast to MDMA, do not cause depletion of cerebral serotonin,[28] we suggest that the observed negative association between MDMA intake and cerebral SERT binding is mediated through a direct presynaptic MDMA effect and a secondary serotonin depletion rather than by the serotonin$_{2A}$ agonistic effects of MDMA. This conclusion is also supported by organotypic hippocampal culture studies showing that the effect of MDMA requires the presence of intact serotonergic terminals.[85] Since most of the drug users in the HPU group had also been using MDMA on 1 or more occasions (with a median of 24 lifetime ingested MDMA tablets), the HPU group could alternatively be considered a group of light to moderate MDMA users. The lack of a difference in SERT binding between the HPU group and the control group is in agreement with results from a comparable group of moderate MDMA users[17] and from a study in light MDMA users.[20]

SEROTONIN$_{2A}$ RECEPTOR BINDING AND USE OF MDMA AND/OR HALLUCINOGENS

Based on the shared stimulatory action on the serotonin$_{2A}$ receptors by MDMA and hallucinogenic compounds, serotonin$_{2A}$ receptor binding was the primary analysis investigated within the entire group of MDMA and hallucinogen users, who together formed the group of serotonin$_{2A}$ agonist users. Compared with the controls, the serotonin$_{2A}$ agonist users had slightly decreased cortical serotonin$_{2A}$ receptor binding. However, this result should be interpreted with caution because the effect was of only borderline significance when 2 control subjects with very high serotonin$_{2A}$ receptor binding levels were regarded as outliers and subsequently excluded from the sample. Also, when MPUs and HPUs were analyzed in separate groups, no significant group difference in serotonin$_{2A}$ receptor binding between MPUs, HPUs, and control subjects was detected. In conclusion, we found slightly decreased cortical serotonin$_{2A}$ receptor binding in serotonin$_{2A}$ agonist users but cannot entirely rule out that this finding was an artifact due to high serotonin$_{2A}$ receptor binding in 2 control subjects.

Stimulation of the serotonin$_{2A}$ receptor has been shown to lead to a transient downregulation of the receptor,[86] and serotonin$_{2A}$ receptor downregulation has also been seen after MDMA administration to rats and use in humans.[33,87] This decrease in serotonin$_{2A}$ receptor binding in rats after MDMA treatment was reversible within 21 days[87]; likewise, in recent ($\leq$3 weeks prior) MDMA users, postsynaptic serotonin$_{2A}$ receptor binding was lower in all cortical areas studied, whereas serotonin$_{2A}$ receptor binding was significantly higher in the occipital cortex of former MDMA ($>$19 weeks prior) users.[33] To determine whether a post hoc analysis could replicate this finding in our group of serotonin$_{2A}$ receptor agonist users, we divided the agonist users into 2 groups consisting of 8 and 10 drug users with periods of abstinence similar to those in the study by Reneman et al.[33] We did not see any between-group difference in serotonin$_{2A}$ receptor binding. The same was the case when we divided the subjects into 2 groups with and without serotonin$_{2A}$ agonist use within the month before the scan, as presented in Figure 5C. The increased serotonin$_{2A}$ receptor binding seen by Reneman et al was interpreted as secondary to low synaptic serotonin levels; the hallucinogen use in our sample could counteract this effect through a compensatory downregulation of serotonin$_{2A}$ receptors. Alternatively, the low SERT binding seen among MDMA users in our study could compensate for an MDMA-induced serotonin depletion, thereby keeping synaptic serotonin at a level where serotonin$_{2A}$ receptors do not upregulate.

A decrease in serotonin$_{2A}$ receptor binding in agonist users could thus represent a transient receptor downregulation secondary to stimulation of the receptor that we were unable to pick up with our limited sample size. Alternatively, a decrease in serotonin$_{2A}$ receptor binding could reflect toxic damage to cortical pyramidal serotonin$_{2A}$ receptor–expressing neurons. In cell cultures, MDMA neurotoxicity can be completely prevented by pretreatment with a serotonin$_{2A}$ receptor antibody, and the normally elicited MDMA depletion of intracellular glutathione can be attenuated by ketanserin, a competitive serotonin$_{2A}$ receptor antagonist.[29,30] The absence of signs indicating that serotonin$_{2A}$ receptor binding changes in this study were reversible could represent permanent damage to the neurons, although there could be other reasons. It could simply be a matter of insufficient power, since the reduction in cortical serotonin$_{2A}$ receptor binding was quite modest.

In a post hoc analysis performed both in the full subject sample (with the exception of the 2 controls with very high serotonin$_{2A}$ receptor binding) and in the group of drug users alone, we replicated a recent finding[62] of a

significant quadratic (inverted U–shaped) relationship between cortical serotonin$_{2A}$ receptor and pallidostriatal SERT binding.

METHODOLOGICAL CONSIDERATIONS

The results should be interpreted in the light of some methodological aspects. For quantification of SERT binding, we used a reference tissue model without arterial blood sampling; thus, the individual nonspecific binding could not be accurately assessed. However, as a proxy for nonspecific binding, we calculated the area under the [^{11}C]DASB cerebellar time-activity curves normalized to the injected dose per kilogram of body weight and found no between-group difference, indicating that differences in nonspecific radiotracer binding did not drive the group differences in cerebral SERT binding.

In addition, when reporting SERT binding from cortical areas with [^{11}C]DASB PET, it should be emphasized that, because of the relatively low SERT binding in these areas, the interindividual variability is high and the signal to noise ratio is low. Consequently, evaluating data from cortical brain regions should be done with caution. However, in a test-retest study using the same method as was used in our study ([^{11}C]DASB PET and MRTM2) except for longer scan time, a high cortical reliability was shown (intraclass correlation coefficients of 0.82 in temporal cortex, 0.85 in occipital cortex, and 0.55 in frontal cortex).[70]

Because of the cross-sectional design of our study, one should consider whether the low cerebral SERT binding in MDMA users was a preexisting trait associated with an increased preference for the use of MDMA. We consider this less likely because, as discussed already, interventional animal studies have shown that MDMA administration leads to decreased cerebral SERT levels, and data from our group and others[17-21,65-67] support the presence of an MDMA dose-response relationship and recovery of SERT binding with abstinence from MDMA.

As in all clinical studies reporting on the consequences of illicit drug use, this study was also prone to difficulties with obtaining reliable and valid data on drug use from the study participants. Not only can it be difficult for participants to remember their exact drug intake in terms of doses and dates, but illicit products often contain drugs other than those presumed. In the present study, we verified the self-reported drug use by measuring drug content in hair samples corresponding to use within the 3 preceding months. In this way, we could confirm the use of MDMA by positive hair test results in 13 of the 14 participants who reported use of MDMA during the same time period, and the one in whom MDMA was not detected reported an intake of only 90 mg of MDMA 36 days before the hair test. Post hoc exclusion of that individual did not change the results. Intake of MDMA in proximity to the PET scans could influence the binding potentials of the radioligands through receptor/transporter blockade. However, by consecutive urine drug assessments, we confirmed an MDMA abstinence period of a minimum of 11 days before the PET scans. That is, we can exclude that the observed changes in cerebral SERT or serotonin$_{2A}$ receptor binding were due to residual amounts of MDMA in the brain.

The content of ecstasy tablets in Denmark is surveyed annually; between 2003 and 2007, 88% of seized tablets contained MDMA as the sole drug in amounts of between 1 and 159 mg (median, 60 mg).[88,89] During the same period, apart from MDMA, 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxyethylamphetamine (MDE), amphetamines were the most commonly found compound in the seized tablets (typically in >7% of seized tablets); 2005 was the year with the highest rate of contaminated tablets (up to 32% of seized tablets contained other compounds). The national reports on seized ecstasy during the study did not include investigations of MDMA in powder form. However, in a more recent report from the Danish Street-Level Project,[90] the purity of MDMA in powder form (<2.5% of MDMA seizures) was determined to be 53% to 78% (pure base). The self-reported use of amphetamine and cocaine was a little higher in the group of MPUs than among the HPUs, and the potential impact of central stimulants on the serotonergic markers should be considered. Use of cocaine, however, has consistently been related to increased cerebral SERT binding[91-93]; therefore, cocaine use is not a likely reason for the decreased SERT binding seen in our study. In contrast, postmortem brain studies[94] and in vivo imaging studies[95] have suggested that use of methamphetamine leads to a reduction in cerebral SERT binding. However, methamphetamine was not present in the hair from our study population, in accordance with methamphetamine being almost absent from the drug market in Denmark. Because of the occasional presence of amphetamine in ecstasy tablets, simultaneous intake of amphetamine was virtually unavoidable, even in users who considered themselves users of MDMA only. However, when recent use of either amphetamine or cocaine was included in the statistical analysis, neither was a significant covariate, and inclusion of these variables in the model did not change the observed effect of MDMA use.

Lifetime and current tobacco smoking was more pronounced among MPUs than among HPUs and controls (Table 3), but it was previously shown in a relatively large sample of healthy humans that there is no correlation between tobacco smoking and cerebral SERT or serotonin$_{2A}$ receptor binding.[45,96] In addition, in a post hoc analysis, inclusion of the number of pack-years or the number of daily cigarettes smoked in the week before the scan as covariates did not change the outcome.

In conclusion, we found evidence that MPUs, but not HPUs, have profound reductions in cerebral SERT binding. Cortical serotonin$_{2A}$ receptor binding was slightly decreased among serotonin$_{2A}$ receptor agonist users (both MPUs and HPUs). We identified a dose-response relationship between lifetime use of MDMA and SERT binding across subjects. Our cross-sectional data also suggest that subcortical, but not cortical, recovery of SERT binding might take place after several months of MDMA abstinence.

Submitted for Publication: March 31, 2010; final revision received January 5, 2011; accepted January 5, 2011.
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Financial Disclosure: None reported.

Funding/Support: The study was sponsored by Rigshospitalet, The Lundbeck Foundation, The Danish Medical Research Council, H:S (Copenhagen Hospital Cooperation) Research Council, Sawmill owner Jeppe Juhl and Wife Ovita Juhls Foundation, and the John and Birthe Meyer Foundation.

Additional Contributions: The authors would like to thank all volunteers for their participation. Bente Hoy, RN, Laerke Damgaard, BS, and Mads Okholm, BS, and the staff in the PET and Cyclotron Unit at Rigshospitalet and in the MR department at Hvidovre Hospital provided invaluable technical assistance. Henrik Rindom, MD, and Susan Tapert, PhD, kindly shared their expertise with regard to subject population and clinical interviews. In addition, the Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health Sciences, University of Copenhagen conducted the hair analyses.

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