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Effects of Cocaine and MDMA Self-Administration on Serotonin Transporter Availability in Monkeys

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Although serotonin (5-HT) can interact with dopamine (DA) systems to modulate the subjective and reinforcing effects of psychostimulants such as cocaine and 3,4-methyldioxymethamphetamine (MDMA, ecstasy), the long-term effects of exposure to psychostimulants on brain 5-HT systems are not well characterized. The present study assessed 5-HT transporter (SERT) availability using positron emission tomography (PET) in rhesus monkeys with the SERT-specific radioligand [^{11}C]3-amino-4-(2-dimethylamino-methyl-phenylsulfanyl)-benzonitrile (DASB). SERT availability was assessed in regions of interest including the caudate nucleus, putamen, anterior cingulate cortex, and cerebellum. [^{11}C]DASB distribution volume ratios (DVRs) were calculated using the cerebellum as the reference region. DVRs were calculated in control monkeys and in cocaine or MDMA self-administering monkeys approximately 24 h after the last self-administration (SA) session. SERT availability did not differ between monkeys with a history of MDMA SA and control monkeys in any region examined. In contrast, monkeys with a history of cocaine SA showed significantly higher levels of SERT availability in the caudate nucleus and putamen compared to control subjects. These results suggest that chronic SA of cocaine, but not MDMA, leads to alterations in serotonergic function in brain areas relevant to drug abuse. The higher level of SERT availability in cocaine-experienced monkeys may lead to a reduced inhibitory tone of 5-HT on the DA system, which may explain, in part, differences in the abuse liability between cocaine and MDMA.
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INTRODUCTION

Cocaine and 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') are commonly abused psychostimulants with similar, yet distinct, mechanisms of action. Cocaine increases synaptic levels of dopamine (DA), serotonin (5-HT), and norepinephrine (NE) via blockade of the presynaptic transporters (DAT, SERT, and NET, respectively; Bennett *et al*, 1995). MDMA also inhibits the uptake of monoamines at the DAT, SERT, and NET (Rothman *et al*, 2001). However, MDMA also serves as a substrate for these monoamine transporters to stimulate nonexocytotic release of DA, 5-HT, and NE (Rudnick and Clark, 1993; Rothman *et al*, 2001). Another difference between cocaine and MDMA is affinity for DAT, SERT, and NET. Cocaine binds with relatively equal affinity to all three transporters (Bennett *et al*, 1995), whereas MDMA has a 10-fold higher affinity for SERT compared to DAT or NET (Steele *et al*, 1987; Battaglia *et al*, 1988). Although cocaine and MDMA both increase DA, which is thought to be the primary mediator of reinforcement (Di Chiara and Imperato, 1988), differences in self-administration (SA) have been reported. For example, MDMA does not maintain rates of responding as high as cocaine in rodents (Ratzenboeck *et al*, 2001) or nonhuman primates (Beardsley *et al*, 1986) under fixed-ratio (FR) schedules. Furthermore, using a progressive-ratio (PR) schedule, Lile *et al* (2005) found that MDMA had lower reinforcing strength than cocaine in nonhuman primates. Thus, behavioral differences in measures of abuse liability exist between cocaine and MDMA.

Increasing brain 5-HT activity can attenuate the behavioral and neurochemical effects of cocaine and MDMA. For example, 5-HT uptake inhibitors have been shown to reduce cocaine and MDMA-induced elevations in extracellular levels of DA in the caudate nucleus (Koch and Galloway, 1997; Czoty *et al*, 2002). SERT inhibitors and substrates have also been shown to decrease cocaine SA in rats and nonhuman primates (Richardson and Roberts, 1991; Kleven and Woolverton, 1993; Czoty *et al*, 2002; Glatz *et al*, 2002) and cue-induced reinstatement (Burmeister *et al*, 2003) in rodents. Clinically, 5-HT uptake inhibitors have been shown

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to attenuate the subjective effects of cocaine (Walsh *et al*, 1994) and MDMA (Tancer and Johanson, 2007). Fenfluramine, a SERT substrate, can also reduce cocaine craving (Buydens-Branchey *et al*, 1998). These studies support an inhibitory role of the 5-HT system on the neurochemical and behavioral effects of cocaine and MDMA.

A critical question for better understanding drug addiction is how the brain changes in response to long-term drug exposure. Evidence suggests that chronic cocaine or MDMA use leads to differential disruptions of brain serotonergic systems, which would suggest different treatment strategies during abstinence. For instance, in studies using single photon emission computed tomography (SPECT), acutely abstinent cocaine-dependent patients had higher SERT binding in striatal brain regions compared to non-drug abusing control subjects (Jacobsen *et al*, 2000). Also, cocaine overdose victims had higher SERT binding in the caudate nucleus and putamen compared to controls (Mash *et al*, 2000). In contrast, compared to controls, MDMA users had lower SERT availability within the caudate nucleus, anterior cingulate cortex (ACC), thalamus, and other cortical regions as measured by positron emission tomography (PET; McCann *et al*, 1998; Buchert *et al*, 2003; Thomasius *et al*, 2003; Buchert *et al*, 2004; McCann *et al*, 2005; Buchert *et al*, 2006). A major caveat of these studies is that human drug abusers frequently have used multiple drugs, which can complicate identification of the precise contributions of cocaine or MDMA to alterations in SERT availability (Gouzoulis-Mayfrank and Daumann, 2006).

The present study was designed to examine SERT availability in the caudate nucleus, putamen, and ACC using PET in nonhuman primates with extensive histories of either cocaine or (±) MDMA SA in comparison with drug-naïve controls responding under a schedule of food reinforcement ($n = 4$ per group). These regions of interest (ROI) were selected based on their association with the reinforcing effects of psychostimulants (Di Chiara and Imperato, 1988; Goldstein and Volkow, 2002). For these studies, the radiotracer [^{11}C]3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile (DASB) was used. [^{11}C]DASB has a higher affinity for SERT compared to DAT and NET and appears to be a more selective and improved radioligand for the SERT compared to [^{11}C]McN5652 (Houle *et al*, 2000; Szabo *et al*, 2002). We hypothesized that SERT availability would be higher in monkeys self-administering cocaine and lower in monkeys self-administering MDMA compared to controls.

METHODS

Subjects and Apparatus

Twelve adult male rhesus monkeys (*Macaca mulatta*) served as subjects. Subjects R-1268, R-1326, R-1346, and R-1427 had self-administered cocaine (Table 1; Czoty *et al*, 2006), but had limited experience self-administering other psychostimulants (Lile *et al*, 2000, 2003, 2005). Subjects R-1361, R-1496, R-1498, and R-1499 had recently self-administered MDMA (Table 1) and subjects R-1523, R-1524, R-1525, and R-1526 were experimentally and drug-naïve at the start of this study. Monkeys in the MDMA and control groups were individually housed in stainless-steel cages with water available *ad libitum* and had visual and auditory contact with each other. Monkeys were fitted with a nylon collar (Primate Products, Redwood City, CA) and trained to sit calmly in a standard primate restraint chair using a specially designed stainless-steel pole that attached to the collar. Experiments were conducted in ventilated and sound-attenuating chambers ($150 \times 74 \times 76$ cm, Med Associates, East Fairfield, VT) designed to accommodate a primate chair (Primate Products). Monkeys were weighed weekly and fed enough food daily (LabDiet Monkey Chow and fresh fruit) to maintain body weights at approximately 95% of free-feeding levels. For the cocaine SA group,

Table 1 Self-Administration Histories (mg/kg) Prior to [^{11}C]DASB PET Studies

	[a]Weekly MDMA intake	Total MDMA intake	[b]Months of SA	Prior (−) cocaine intake
MDMA group				
R-1361	22.2	107.46	6	118.33
R-1496	6.2	138.44	18	69.75
R-1498	6.8	96.63	8	80.01
R-1499	3.9	141.74	16	0.2
Mean (±SEM)		121.07 (13.0)	12 (3.4)	67.07 (28.4)
	[a]Weekly cocaine intake	**Total cocaine intake**	**[b]Months of SA**	**Prior (±) MDMA intake**
Cocaine group				
R-1268	1.8	1028.79	10	0.00
R-1326	1.83	537.27	12	129.55
R-1346	24	645.3	5	40.61
R-1427	2.19	759.04	14	0.00
Mean (±SEM)		742.60 (121.9)	10.25 (2.2)	42.54 (35.3)

[a]Weekly intake (mg/kg) refers to drug intake over the 5 days before the PET study.
[b]Months of SA refers to the number of months each subject had been exclusively self-administering either MDMA or cocaine.

monkeys were individually housed in sound-attenuating chambers (91 cm^3; Plas Labs, Lansing, MI). The front wall of each cubicle was constructed of Plexiglas to allow the monkey visual access to the laboratory. Each cubicle was equipped with two response levers (BRS/LVE, Beltsville, MD); only the right lever was used in the present studies. Four stimulus lights, alternating white and red, were located in a horizontal row above each lever. Each animal was fitted with a stainless-steel restraint harness and spring arm (Restorations Unlimited, Chicago, IL) that attached to the rear of the cubicle. Monkeys were weighed monthly and fed enough food daily (LabDiet Monkey Chow and fresh fruit) to maintain body weights at approximately 95% of free-feeding levels and had water available *ad libitum*. All procedures were performed in accordance with established practices as described in the National Institutes of Health Guide for Care and Use of Laboratory Animals. In addition, all procedures were reviewed and approved by the Animal Care and Use Committee of Wake Forest University. Environmental enrichment was provided as outlined in the Animal Care and Use Committee of Wake Forest University Non-Human Primate Environmental Enrichment Plan.

All eight monkeys in the SA groups had been exposed to cocaine at some time in their history (Table 1). However, monkeys in the cocaine SA group had an average lifetime intake 10 times higher than monkeys in the MDMA group (approximately 743 *vs* 67 mg/kg; Table 1). In addition, monkeys in the MDMA SA group had not self-administered cocaine for at least 9 months prior to the PET-imaging studies. Monkeys in the MDMA group had mean MDMA intakes of 121.07 mg/kg; range of intakes was 100–140 mg/kg (Table 1). On average, monkeys in the cocaine and MDMA groups had been self-administering the primary drug of interest for 10 and 12 months, respectively (Table 1).

Catheter Implantation

Under sterile conditions, each monkey was surgically prepared with an indwelling intravenous catheter and vascular access port (Access Technologies, Skokie, IL), placed into a major vein (internal or external jugular, femoral or brachial) as previously described (Czoty *et al*, 2006).

Self-Administration Conditions

Three groups of monkeys ($n = 4$/group) were studied: cocaine SA, MDMA SA, and food-reinforced control monkeys. For monkeys in the cocaine SA group, cocaine doses (saline, 0.03–0.3 mg/kg/injection) were made available, in random order, under a PR schedule of reinforcement as described previously (Czoty *et al*, 2006). Briefly, under the PR schedule, white lights were illuminated above the right lever and 50 responses resulted in a 10-s injection, extinguishing of white lights and illumination of red lights for 10 s. A 10-min timeout (TO) period, during which no lights were illuminated and responding had no scheduled consequences, followed each injection. The response requirement for subsequent injections was determined by the exponential equation used by Richardson and Roberts (1996): ratio $= 5 \times$ exponent $(S^R\# \times 0.2) - 5$. Sessions ended when 2 h elapsed without an injection. For monkeys in the MDMA SA group, responding was maintained under a concurrent FR 30 schedule of MDMA and food pellet presentation. Briefly, 30 consecutive responses on one lever (counterbalanced across monkeys) activated the infusion pump, resulting in an MDMA injection; 30 consecutive responses on the other lever resulted in presentation of a banana-flavored food pellet. Each completion of the FR requirement was followed by a 30-s TO. If a response was made on the alternate lever before an FR 30 was completed, the response requirement was reset. The session ended after 30 total reinforcers had been earned or 60 min had elapsed. MDMA doses (saline, 0.03–0.3 mg/kg/injection) were examined in random order for at least five consecutive sessions and until responding was deemed stable (% injection-lever responding $\pm$ 20% of the mean of three consecutive sessions with no trend). For monkeys in the control group, responding was maintained under an FR 30 schedule of food pellet presentation; a 30-s TO followed each food presentation and sessions ended after a maximum of 30 reinforcers or after 1 h.

MR and PET Imaging

Magnetic resonance imaging (MRI) scans were acquired for each monkey. Approximately 20 min before the MRI, subjects were anesthetized with ketamine (10 mg/kg, i.m.) and transported to the MRI facility. Anesthesia was maintained during the scanning procedure with ketamine supplements when necessary. T1-weighted images of the entire brain were acquired with a 1.5-Tesla GE Signa NR scanner (GE Medical Systems). Images were used to anatomically define ROIs, including the caudate nucleus, putamen, ACC, and cerebellum, for later registration with PET images.

On the day of a PET study, no behavioral experiments were conducted. Prior to the start of the PET study, monkeys were anesthetized with 8 mg/kg ketamine and intubated. Anesthesia was maintained throughout the scan by inhaled isoflurane (1.5%). A Catheter (22-gauge angiocath; Becton Dickinson Vascular Access, Sandy, Utah, USA) was placed in an external vein by percutaneous stick for administration of [^{11}C]DASB at the start of the scan and delivery of lactated ringer's solution (i.v.) to the monkey throughout the study. A paralytic (0.07 mg/kg vecuronium bromide) was administered i.v. and respiration was maintained by a ventilator. Supplemental doses of vecuronium bromide (0.1 mg/h) were administered throughout the study. [^{11}C]DASB was injected at the start of the scan, followed by 3 ml heparinized saline. The average dose injected and specific activity for each group was: control, 8.3 mCi and 1090 ± 386 mCi/μmole; MDMA, 8.5 mCi and 676 ± 140 mCi/μmole; cocaine, 7.5 mCi and 3189 ± 943 mCi/μmole.

Each subject was imaged for 90 min from the time of radioligand injection on a GE Advance NXi PET Scanner (General Electric Systems, Milwaukee, WI). This device provides 35 contiguous transverse slices with a 4.25 mm center-to-center spacing over a 15.2 cm axial field of view (DeGrado *et al*, 1994). The first five frames of each study's PET image data were then added together. This summed

image represents tracer uptake in the early part of the study and approximates a blood flow image. This image was then registered to the animal's MRI using the AIR algorithm (Woods *et al*, 1993) after extracting the brain image from the MRI using the method of Smith (2002). This method provides excellent registration of cortical and subcortical regions. Spherical ROIs for the caudate nucleus, putamen, ACC, and cerebellum were then drawn on each subject's MRI and transferred to their coregistered PET scans. Time–activity curves were generated and distribution volumes ratios (DVRs) were calculated using the cerebellum, a region relatively devoid of SERT, as the reference region and the graphical method of Logan *et al* (1996). This method has previously been shown to provide valid, reproducible measures of SERT binding potentials with low variance between subjects (Meyer *et al*, 2004). For all regions, DVRs from the right and left sides were not statistically different and were averaged.

Statistical Analysis

The primary dependent variable examined was the DVR for each ROI (caudate nucleus, putamen, and ACC). DVRs across all three experimental groups were analyzed using a one-way ANOVA with a *post hoc* Tukey's test for each ROI. In all cases, differences were considered significant at the 95% level of confidence ($P<0.05$).

RESULTS

The mean weekly intakes of MDMA ranged from approximately 4.0 to 22 mg/kg, and for cocaine ranged from approximately 2.0 to 24 mg/kg (Table 1). Individual and group mean DVRs are shown in Table 2. ANOVA revealed a main effect of SA history in the caudate nucleus ($F_{2,9}=10.99$, $P<0.01$), putamen ($F_{2,9}=6.34$, $P<0.05$), and ACC ($F_{2,9}=4.84$, $P<0.05$). *Post hoc* analysis indicated significantly higher SERT availability in monkeys from the cocaine SA group compared to both MDMA SA and control monkeys in the caudate nucleus and putamen ($P<0.05$; Figure 1). There were no statistically significant differences between monkeys in the MDMA SA group and control subjects in these brain regions. In the ACC, SERT availability was significantly higher in the cocaine SA group compared to the MDMA SA group ($P<0.05$). In the ACC, mean DVRs were not different from the control group in either cocaine or MDMA SA monkeys.

Table 2 Individual and Group DVRs for Each Region of Interest

	Caudate nucleus	Putamen	Anterior cingulate cortex
Control group			
R-1523	1.8	2.0	1.46
R-1524	1.39	1.36	1.19
R-1525	1.92	1.97	1.43
R-1526	1.61	1.82	1.31
Mean (±SEM)	1.68 (0.12)	1.79 (0.15)	1.35 (0.06)
MDMA group			
R-1361	1.43	1.63	1.18
R-1496	1.69	1.83	1.28
R-1498	1.48	1.62	1.18
R-1499	1.5	1.77	1.17
Mean (±SEM)	1.53 (0.06)	1.71 (0.05)	1.20 (0.03)
% Difference	−9.2 (3.4)	−4.3 (2.9)	−10.9 (1.9)
Cocaine group			
R-1268	2.05	2.43	1.44
R-1326	2.26	2.46	1.89
R-1346	2.02	2.21	1.51
R-1427	1.94	2.02	1.35
Mean (±SEM)	2.07 (0.07)	2.21 (0.09)	1.55 (0.12)
% Difference	23.1 (4.1)	27.4 (5.8)	14.6 (8.8)

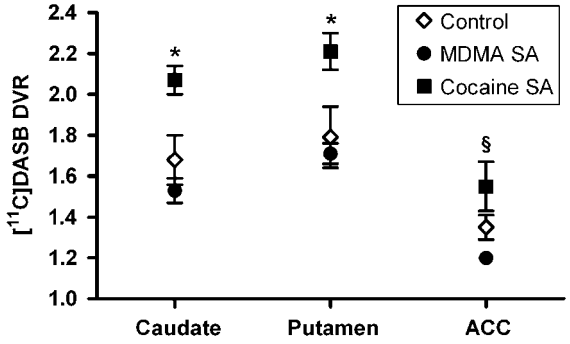

Figure 1 SERT availability in the caudate nucleus, putamen and anterior cingulate cortex (ACC) in monkeys with extensive histories of cocaine or MDMA self-administration (SA) or food reinforcement (Control). Data are shown as mean (±SEM) distribution volume ratios (DVR) for each group ($n=4$). *Indicates significantly different from control and MDMA SA groups ($P<0.05$). §Indicates significantly different from MDMA SA group only ($P<0.05$).

DISCUSSION

The goal of the present study was to compare SERT availability in monkeys with histories of cocaine or MDMA exposure to drug-naïve control subjects using PET imaging. Compared to controls, monkeys with long-term cocaine SA histories had significantly higher levels of SERT availability in the caudate nucleus and putamen. In contrast, levels of SERT availability in monkeys with long-term MDMA SA histories were not significantly different from control subjects. These data provide evidence for differences in the neuropharmacological consequences of long-term cocaine and MDMA exposure, which may explain differences in the reinforcing strength of cocaine compared to MDMA (Lile *et al*, 2005).

In the present study, a history of cocaine SA was associated with higher levels of SERT, extending preclinical rodent studies (Cunningham *et al*, 1992) to nonhuman primates and to PET imaging. Acutely abstinent cocaine-dependent patients (Jacobsen *et al*, 2000) or cocaine overdose victims (Mash *et al*, 2000) also had higher SERT binding in anterior and posterior striatal regions compared

to controls. Interestingly, these higher levels of SERT parallel the effects of cocaine on DAT densities reported in monkeys with long-term cocaine SA histories (Letchworth *et al*, 2001) and humans (Little *et al*, 1993). The present results are consistent with the hypothesis that long-term cocaine exposure may lead to a reduced 5-HT tone due to increased SERT function. However, randomized clinical trials have failed to show any effectiveness of 5-HT uptake inhibitors alone in treating cocaine dependence (Grabowski *et al*, 1995; Schmitz *et al*, 2001). Since both DAT and SERT are higher after long-term cocaine SA histories in nonhuman primates (Letchworth *et al*, 2001; present study) and humans (Little *et al*, 1993; Mash *et al*, 2000), pharmacotherapies that target both these monoamine transporters might be more efficacious for treating cocaine dependence (cf. Rothman and Baumann, 2003; Howell *et al*, 2007).

In contrast to cocaine, long-term MDMA SA did not affect SERT availability. Human imaging studies involving active ecstasy abusers and controls have reported conflicting results regarding SERT availability in brain regions similar to those examined in the present study. Two radioligands, [^{11}C]McN5652 and [^{11}C]DASB, have been used to examine SERT availability in MDMA users. Studies using [^{11}C]McN5652 have reported either decreases (McCann *et al*, 1998; Buchert *et al*, 2003; Thomasius *et al*, 2003; Buchert *et al*, 2004) or a lack of effect (Buchert *et al*, 2006; Thomasius *et al*, 2006) in SERT availability in the caudate nucleus, putamen, or ACC. McCann *et al* (2005) compared [^{11}C]McN5652 and [^{11}C]DASB in the same MDMA users and found a high correlation and no significant differences between the radiotracers. That study reported significantly lower levels of SERT availability in the ACC, but not the caudate or putamen, compared to control subjects. Thus, the results of the present study are consistent with data from human subjects demonstrating no effect of MDMA on SERT availability in the caudate nucleus or putamen (McCann *et al*, 2005). Importantly, the present study demonstrated no effect of MDMA under conditions in which a history of cocaine exposure resulted in higher levels of SERT availability compared to controls.

Because SERT availability is influenced by both numbers of transporters and levels of competition from endogenous 5-HT, the functional tone of the serotonergic system is difficult to determine using PET data alone. There is a paucity of studies examining the effects of endogenous 5-HT on DASB binding. *In vitro* evidence suggests that at relatively high concentrations, 5-HT can inhibit DASB binding (Hummerich *et al*, 2004). In contrast, *in vivo* evidence for 5-HT competing with DASB at the SERT is conflicting (Ginovart *et al*, 2003; Milak *et al*, 2005; Praschak-Rieder *et al*, 2005; Talbot *et al*, 2005). Thus, the influence of endogenous levels of 5-HT on differences in SERT availability between cocaine SA monkeys and monkeys from the MDMA or control groups cannot be ruled out. Future studies using *in vivo* microdialysis and PET imaging examining 5-HT levels in nonhuman primates self-administering cocaine and MDMA could help explain how chronic drug exposure alters 5-HT levels and influences [^{11}C]DASB measures.

Previous research in nonhuman primates treated with MDMA (5 mg/kg, twice daily for 4 days) showed acute (9–40 days) reductions in SERT availability, as measured by PET, in the caudate nucleus, putamen, and cortical regions, with decreases in cortical regions still apparent 13 months after MDMA (Scheffel *et al*, 1998; Szabo *et al*, 2002). Rhesus monkeys treated with a similar dosing regimen as in Scheffel *et al* (1998) also showed acute (4–31 days) decreases in SERT density as measured by SPECT (Reneman *et al*, 2002). Nonetheless, monkeys from the present study had only modest, nonsignificantly lower SERT availability (approximately 7%) compared to controls. Reasons for the discrepancy between the earlier reports and the present study may include differences in MDMA dose and regimen. In the studies involving nonhuman primates showing lower SERT availability, MDMA was administered by the experimenter (5 mg/kg, s.c., twice daily for 4 consecutive days) rather than self-administered as in the present study (average total session intake ranged from 0.3 to 2.3 mg/kg dependent upon the dose available). Contingent *vs* noncontingent drug administration can profoundly influence the behavioral and neurochemical effects of drugs (Dworkin *et al*, 1995; Hemby *et al*, 1997). In fact, Fantegrossi (2007) has argued that the noncontingent, experimenter-administered dosing regimens employed in the preclinical studies described above do not model the human abuse condition and that the model of intravenous self-administration is more appropriate for determining the neurochemical consequences of MDMA exposure. Moreover, using 'effect scaling' to determine the dose administered to rats, MDMA does not significantly decrease SERT protein expression (Wang *et al*, 2004, 2005). Therefore, it is possible that the method and dosage of MDMA administration may have contributed to discrepancies between present and previous observations. The use of MDMA doses that are more analogous to humans based on self-administration studies (present study; Fantegrossi *et al*, 2004) or using effect scaling (Wang *et al*, 2004, 2005) might be more appropriate in ascertaining the neurochemical consequences of MDMA.

Another potential reason for statistically insignificant differences between monkeys in the MDMA SA group and control subjects observed in our study may relate to the amount of drug exposure or that the present study was not a within-subjects design. One advantage of using nonhuman primates is the ability to perform multiple scans within subjects over prolonged periods of time which was not used in the present study due to experimental constraints. It is possible, although unlikely (see Fantegrossi *et al*, 2004), that greater intakes of MDMA than were self-administered in the present study are necessary for neuroadaptations to occur (see Table 1).

It should also be mentioned that although all subjects had been self-administering either cocaine or MDMA for at least 6 months prior to assessing SERT availability, previous exposure to other drugs could be a confound. However, previous preclinical and clinical studies would argue against this point since differences were reported in cocaine-exposed rodents (Cunningham *et al*, 1992) and humans (Jacobsen *et al*, 2000; Mash *et al*, 2000). Also, one potential reason why we did not detect significantly lower levels of SERT availability in select ROI in the monkeys self-administering MDMA could be related to their prior exposure to cocaine, which may have increased SERT availability prior to the predicted MDMA-induced

reductions. This might also explain why Fantegrossi *et al* (2004) did not observe lower 5-HT neurochemical markers since those monkeys were 'co-abusing' MDMA and cocaine. Therefore, future longitudinal, PET-imaging studies are warranted to address the issue of how cocaine-induced changes in SERT are affected by subsequent MDMA exposure.

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DISCLOSURE/CONFLICT OF INTEREST

We declare that there is no conflict of interest for any of the authors.

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