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Validity of [^{123}I]β-CIT SPECT in Detecting MDMA-Induced Serotonergic Neurotoxicity

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ABSTRACT Recent [^{123}I]β-CIT single-photon emission computed tomography (SPECT) studies revealed decreased serotonin transporters (SERT) density in the brain of humans with a history of MDMA ("Ecstasy") use. However, [^{123}I]β-CIT SPECT has until now not been validated as a method for detecting such serotonergic lesions. Therefore, the present study was undertaken. Following baseline [^{123}I]β-CIT SPECT scans, a rhesus monkey was treated with MDMA (5 mg/kg, s.c. twice daily for 4 consecutive days). SPECT studies 4, 10, and 31 days after MDMA treatment revealed decreases in [^{123}I]β-CIT binding ratios in the SERT-rich brain region studied (hypothalamic/midbrain region), with SERT density reduced by 39% in this brain region 31 days after treatment. Data obtained with SPECT studies correlated well with SERT density determined with autoradiography after sacrifice of the animal (−34%). In addition, ex vivo [^{123}I]β-CIT binding studies in rats 1 week after treatment with neurotoxic doses of MDMA (20 mg/kg s.c. twice daily for 4 consecutive days) revealed significant reductions in [^{123}I]β-CIT binding in SERT-rich regions (including the hypothalamus) when compared to saline-treated rats. The combined results of these studies indicate that SPECT imaging of SERT with [^{123}I]β-CIT can detect changes in SERT density secondary to MDMA-induced neurotoxicity in the hypothalamic/midbrain region, and possibly other brain regions. **Synapse 46:199–205, 2002.**

INTRODUCTION

Alterations of serotonin (5-HT) transmission have been implicated in numerous neuropsychiatric disorders. 5-HT transporters (SERTs), located on presynaptic terminals, terminate the action of 5-HT by reuptake into the presynaptic neuron. Because the SERT is a structural component specifically located on 5-HT terminals and cell bodies, SERT density can serve as a marker of the number or integrity of 5-HT nerve terminals. Thus, the ability to measure 5-HT transporters in vivo with positron emission tomography (PET) or single-photon emission computed tomography (SPECT) is critical in characterizing putative abnormalities of 5-HT neurons in neuropsychiatric disorders.

Imaging of the SERT has been employed to investigate the potential neurotoxicity of the popular recreational drug 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy") in the human brain (McCann et al., 1998; Semple et al., 1999; Reneman et al., 2001a,b). Although generally regarded as relatively safe, it has become increasingly apparent that MDMA use can lead to toxic effects on brain 5-HT neurons in rodents and nonhuman primates. Damage to 5-HT neurons has been demonstrated by reductions in various markers unique to 5-HT axons, including brain 5-HT, 5-hydroxyindoleacetic acid, and the density of SERTs (Battaglia et al., 1987; Commins et al., 1987; Schmidt, 1987; Schmidt et al., 1986; Stone et al., 1986).

Few data are available on the neurotoxic effects of MDMA in the human brain. Since SPECT is widely available at relatively low cost, it could greatly contribute to our understanding of MDMA's short- and long-term effects in the human brain. Recent [^{11}C]McN 5652

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PET and [^{123}I]β-CIT SPECT studies have shown decreases in the number of central SERTs in humans with a history of MDMA use (McCann et al., 1998; Semple et al., 1999; Reneman et al., 2001a,b). Whereas the use of [^{11}C]McN 5652 PET in detecting MDMA-induced 5-HT neurotoxic lesions has been validated (Scheffel et al., 1998; Szabo et al., 2002), the use of ^{123}I-labeled β-CIT SPECT has until now not been validated to detect such 5-HT lesions.

The present study was undertaken in an effort to validate the use of [^{123}I]β-CIT SPECT to visualize and quantify regional changes in SERT in the living monkey brain following MDMA exposure. The suitability of [^{123}I]β-CIT for the detection of serotonergic neuronal damage was also evaluated using ex vivo [^{123}I]β-CIT binding studies in rat brain.

MATERIALS AND METHODS

Animals and drug treatment

(±)Methylenedioxymethamphetamine hydrochloride (certified reference compound, purity >98.9%) was obtained from the Netherlands Forensic Institute (Rijswijk, The Netherlands). ^{123}I labeling of β-CIT was performed by oxidative radioiododestannylation (Radionuclide Center, Vrije University, Amsterdam, The Netherlands) of the trimethylstannyl precursor obtained from RBI (Natick, MA). [^{123}I]β-CIT had a specific activity >185 MBq/nmol and a radiochemical purity >97%.

All experiments involving procedures using animals were approved by the local Animal Care Committee. For the in vivo experiment a male rhesus monkey (*Macaca mulatta*) was used, which had been in a previous experiment in which neuronal synchronizations after visual stimulation of the occipital cortex were recorded with electrodes. SPECT studies were on five different occasions, twice before and three times after treatment with MDMA. Control baseline studies were carried out over a 3-week period; the post-MDMA treatment studies were performed 4, 10, and 31 days after administration of the last dose of MDMA hydrochloride. Dissolved in saline, MDMA was injected subcutaneously in doses of 5 mg/kg twice a day for 4 days. One male rhesus monkey served as control for autoradiography.

Male Wistar rats (obtained from Broekman Institute B.V., Someren, The Netherlands) weighing 200–250 g were used in ex vivo [^{123}I]β-CIT binding studies. Groups of rats (n = 4–5) were given either a vehicle or a neurotoxic regimen of MDMA which consisted of a subcutaneous dose of 20 mg/kg MDMA given twice a day subcutaneously for 4 consecutive days. [^{123}I]β-CIT binding analysis was performed 7 days after the last treatment.

In vivo SERT imaging in monkey brain

One day before SPECT scanning the monkey received potassium iodide orally in order to prevent thyroid uptake of free radioactive iodide. During the scanning procedure, the monkey was anesthetized by an intramuscular injection of a mixture of ketamine-HCl (0.1 mg/kg), acepromazine maleate (0.02 mg/kg), and atropine sulfate (0.05 mg/kg). Anesthesia was maintained during the experiment with hourly injections of ketamine (0.1 mg/kg). SPECT studies were performed using the Strichman Medical Equipment 810X tomographic system. This 12-detector single-slice scanner has a full-width at half-maximum resolution of approximately 7.5 mm. Each acquisition consisted of at least nine slices (acquired in a 64 × 64 matrix), 3 min per slice (interslice distance 5 mm). The energy window was set at 135–190 keV. Acquisition was started 2 and 3 h after intravenous injection of approximately 222 MBq [^{123}I]β-CIT (specific activity >185 MBq/nmol; radiochemical purity >98%), when equilibrium at the SERT was approached (Laruelle et al., 1993). To ensure reproducible positioning of the monkey's head in the camera, a headholder was used. The head of the monkey was oriented parallel to the canthomeatal (CM)-line with the help of beams from gantry-mounted lasers. Attenuation and reconstruction correction of the SERT images were performed as described previously (Booij et al., 1997).

Image analysis was performed using a region of interest (ROI) analysis. ROIs for the hypothalamic/midbrain region, striatum, and cerebellum were used. We defined the hypothalamic/midbrain region as 10 mm below the level of the basal ganglia and the cerebellar level 20 mm below the level of the basal ganglia. Following this, by reference to the activity distribution, a neuroanatomical rhesus monkey atlas and co-registered MRI scans were used to reassure that the ROI was correctly placed. MR images were obtained at 1.5T with a Siemens Magnetom SP 63 SP/4000 scanner. Because electrodes had been placed in a previous experiment in the occipital and parietal cortex of the rhesus monkey, we did not analyze neocortical brain regions. The measured concentration of radioactivity was expressed as Strichman Medical Units SMUs; 1 SMU = 100 Bq/ml (as specified by Strichman Medical Equipment, Medfield, MA). The uptake in the cerebellum, presumed free of SERT, was used as a reference for background radioactivity. Binding of [^{123}I]β-CIT in the hypothalamic/midbrain region and striatum was analyzed using the ratio of specific (=total binding in the ROI—nonspecific binding) to nonspecific binding (=binding in cerebellum). Consequently, specific to nonspecific binding ratios of >0 indicate specific binding.

Autoradiography in monkey brain

Three weeks after the last SPECT experiment, the rhesus monkey was sacrificed with an i.v. overdose of pentobarbital. An additional rhesus monkey was used as a control. The brains were immediately dissected on

TABLE I. SERT binding in rhesus monkey

	[123I]β-CIT SPECT[1]			[3H]Citalopram[2]		
	pre-MDMA[3]	post-MDMA 31 days[4]	delta (%)	Control	post-MDMA 52 days[4]	delta (%)
Hypothalamic/midbrain	1.68	1.11	−34	8.10	5.03	−38
Striatum	14.53	10.79	−26	2.40	1.83	−24

[1]Expressed as specific to nonspecific binding ratios.
[2]Mean of 3–4 sections expressed in nCi/mg of tissue.
[3]Mean of two observations.
[4]Days after last MDMA injection.

ice and samples stored at −80°C until processed. Autoradiographic studies of SERT were carried out using [3H]citalopram as previously described (Fischer et al., 1995).

Ex vivo SERT binding studies in rat brain

MDMA and saline-treated rats were injected i.v. with approximately 1.85 MBq [123I]β-CIT 7 days after the last treatment. Three hours after injection of [123I]β-CIT (Reneman et al., 1999), animals were killed by bleeding via heart puncture under ether anesthesia. The brains were quickly removed and dissected into the following regions: prefrontal cortex, temporal cortex, parietal cortex, occipital cortex, hippocampus, striatum, thalamus, raphe nuclei, midbrain (containing raphe nuclei, substantia nigra, and superior colliculi), and cerebellum and weighed. 123I radioactivity of [123I]β-CIT in each region was assayed with a gamma counter. The data were corrected for radioactivity decay back to the time of preparation of the injection syringes in order to compare relative concentrations in the tissues taken and to relate the results to the injected dose. The amount of radioactivity was expressed as a percentage of the injected dose, multiplied by the body weight per gram tissue weight (% ID × kg/g tissue), as described previously (Rijks et al., 1996). As in the SPECT experiment, the uptake in the cerebellum, presumed free from SERT, was used as a reference for background radioactivity. Binding of [123I]β-CIT to SERT was analyzed using the ratio of specific to nonspecific binding.

Statistics

Statistical significance of the ex vivo binding studies in rats were evaluated using Student's *t*-tests. The criterion for significance was $P < 0.05$.

RESULTS

In vivo SERT imaging in monkey brain

Hypothalamic/midbrain [123I]β-CIT binding ratios were reduced after treatment with MDMA compared to the nontreated situation. SERT densities were lowest 4 days after MDMA treatment. On day 31 (last SPECT measurement) SERT densities were higher compared to days 4 and 10. [123I]β-CIT binding ratios obtained 10 days after MDMA treatment in the hypothalamic/midbrain region were reduced by 39% compared to the nontreated situation. After 31 days this was 34% (Table I, Figs. 1, 2). Striatal [123I]β-CIT binding ratios were reduced by 13% after 10 days and 26% after 31 days.

The reproducibility of the SPECT measurements seems to be good. This is demonstrated by the relatively small variations in mean [123I]β-CIT binding ratios obtained on two different occasions before MDMA treatment (4% for the hypothalamic/midbrain region and 9% for the striatum).

There was good agreement between the in vivo SPECT data and [3H]citalopram binding of the control and MDMA-treated rhesus monkeys in the hypothalamic/midbrain region and striatum (Table I).

Ex vivo SERT binding studies in rat brain

Significant reductions in specific binding ratios of [123I]β-CIT occurred after treatment with MDMA (Table II). Statistically significant decreases in [123I]β-CIT binding were especially prominent in areas of high concentrations of SERT but low concentrations of dopamine transporters. For instance, the decrease in the hypothalamus was 55% from controls, 57% in the midbrain, 59% in the thalamus, and 74% in the occipital cortex. On the other hand, in the striatum [123I]β-CIT binding ratios increased nonsignificantly by 19%. [123I]β-CIT binding ratios were not significantly reduced in the raphe nuclei of MDMA-treated rats compared to controls (Table II).

DISCUSSION

In the present study, we examined the validity of [123I]β-CIT SPECT in detecting MDMA-induced serotonergic neurotoxicity. Treatment of rats with neurotoxic doses of MDMA led to significant reductions in [123I]β-CIT binding in SERT-rich regions. Using [123I]β-CIT SPECT, comparable observations were made in an MDMA-treated monkey. Autoradiography showed clear loss of SERT in the MDMA-treated monkey as compared to the control monkey. These results indicate that [123I]β-CIT SPECT is capable of detecting MDMA-induced 5-HT neurotoxic lesions in the hypothalamic/midbrain region.

In a previous study, [11C]β-CIT PET was not found to be a suitable technique to detect neurotoxic effects of

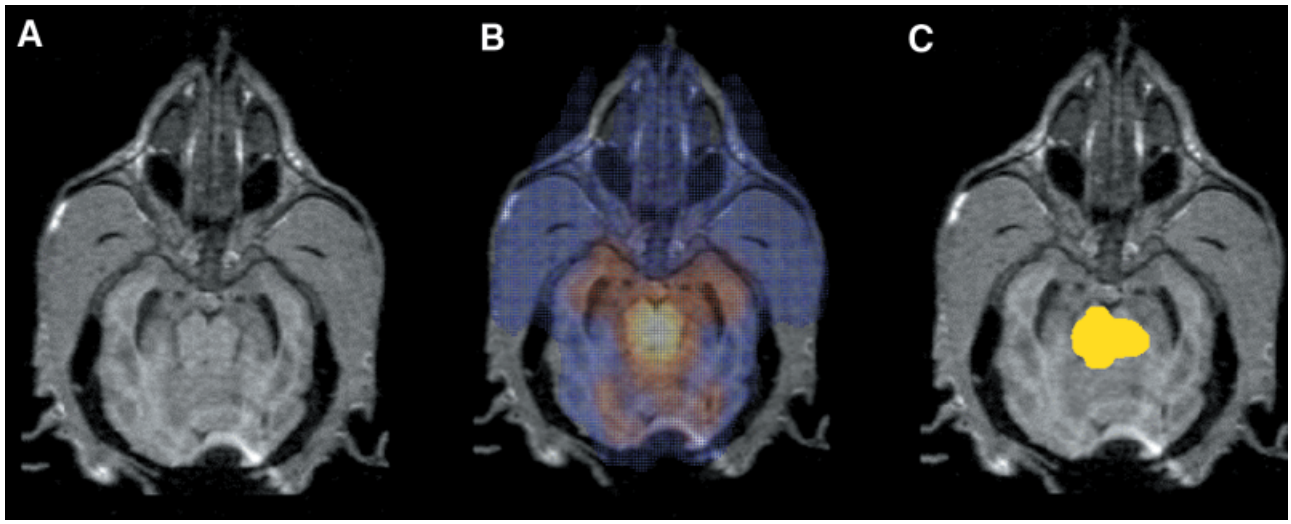

Fig. 1. **A:** Transverse MR image at the level of the hypothalamic/midbrain region. Multislice 3D sagittal images (80 slices; 1 mm) were obtained using gradient-echo imaging (TR/TE; 9.7/4 ms, 256 mm FOV, 256 × 256 matrix). Transverse and coronal slices were reconstructed from the sagittal series. **B:** Co-registration image from (**A**) and SPECT image at the same level, showing uptake of [^{123}I]β-CIT in the hypothalamic/midbrain region. Co-registration of MR and SPECT images was performed using the Hermes Multi Modality software package (Nuclear Diagnostics, Stockholm, Sweden). **C:** Voxel analysis comparing mean specific [^{123}I]β-CIT binding ratios in a rhesus monkey (as measured with SPECT) before (n = 2) and after treatment with MDMA (n = 3), displayed on a co-registered MR image. After registration in the same orientation an unpaired Student's *t*-test was performed on each voxel of generated SPECT images. The yellow region represents significant lower ($P < 0.025$) specific [^{123}I]β-CIT binding ratios after MDMA treatment compared to the nontreated situation in the hypothalamic/midbrain region. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

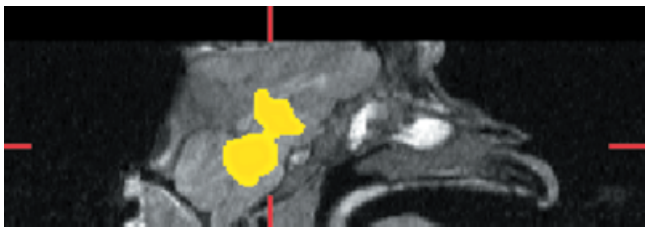

Fig. 2. Voxel analysis as in Figure 1C on sagittal MR image. The yellow region represents significantly lower ($P < 0.025$) specific [^{123}I]β-CIT binding ratios after MDMA treatment compared to the nontreated situation in the hypothalamic/midbrain region and thalamus. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II. Specific [^{123}I]β-CIT SERT binding ratios in saline and MDMA-treated rats[1]

	Control	MDMA	delta (%)	*P*
Prefrontal cortex	1.94 ± 0.58	0.71 ± 0.20	−63	< 0.01
Temporal cortex	1.27 ± 0.33	0.57 ± 0.33	−55	0.01
Parietal cortex	0.86 ± 0.15	0.30 ± 0.08	−65	< 0.01
Occipital cortex	1.41 ± 0.21	0.37 ± 0.08	−74	< 0.01
Amygdala	2.82 ± 0.65	1.38 ± 0.82	−51	0.015
Hippocampus	1.29 ± 0.48	0.60 ± 0.05	−53	0.012
Striatum	14.1 ± 2.74	16.8 ± 2.37	+19	0.135
Thalamus	3.49 ± 0.56	1.43 ± 0.28	−59	< 0.01
Hypothalamus	3.34 ± 0.84	1.51 ± 0.42	−55	< 0.01
Raphe nuclei	3.57 ± 0.84	2.63 ± 0.96	−26	0.142
Midbrain	2.68 ± 0.41	1.15 ± 0.44	−57	< 0.01

MDMA, since no significant changes in [^{11}C]β-CIT binding before and after MDMA administration were observed in baboon brain (Scheffel et al., 1998). As pointed out in that article, this is probably due to the low separation between specific and nonspecific binding because of the short physical half-life (20 min) of the ^{11}C-labeled tracer and the slow kinetics of [^{11}C]β-CIT. By contrast, we here demonstrate that the ^{123}I-labeled version of the radiotracer is capable of detecting MDMA-induced neurotoxic effects in 5-HT-rich brain regions, both in ex vivo binding assays and in vivo SPECT. The half-life time of ^{123}I (6.2 h) offers the opportunity to await separation between specific and nonspecific binding. These findings confirm and extend previous in vitro and ex vivo studies in which [^{125}I]β-CIT was found to adequately detect MDMA and fenfluramine induced 5-HT neurotoxic lesions (Lew et al., 1996; Scheffel et al., 1992).

The observed decreases in hypothalamic/midbrain SERT densities in rat and monkey brain after MDMA treatment very likely reflects MDMA-induced brain 5-HT neurotoxicity, since numerous other studies have also documented reductions in SERT densities in animals with known MDMA-induced 5-HT injury, while leaving dopamine and dopamine transporter densities unaffected (Battaglia et al., 1987; Commins et al., 1987). It should be kept in mind, however, that it is an assumption that a decrease in SERT density directly reflects axonal loss. Several factors, such as internalization of the SERT, could also result in decreased binding. In this respect, the lower SERT values observed in the SPECT study at 4 days when compared to

day 10 after treatment with MDMA may be related to SERT internalization rather than MDMA-induced loss of SERT, whereas at 10 days and 31 days the pharmacologic effects of MDMA are negligible and SERT reductions more likely reflect SERT loss. The half-life estimates for (S)-(+) and R-(-)-MDMA in rats are 73.8 and 100.7 min, respectively (Cho et al., 1990). Because [123I]β-CIT binding studies in rats were performed 7 days after MDMA treatment and in the monkey were started after 4 days, it is unlikely that MDMA administration would have caused acute pharmacological effects and interfered with [123I]β-CIT binding. It has been shown that other 5-HT axonal markers are also reduced after MDMA treatment (Sabol et al., 1996). Furthermore, correlative anatomic studies indicate that loss of presynaptic SERT in MDMA-treated animals is related to damage of 5-HT axons and axon terminals (Battaglia et al., 1987; Commins et al., 1987; Fischer et al., 1995). Consequently, it can be concluded that the observed decrease in [123I]β-CIT binding in the hypothalamic/midbrain region reflects degeneration of 5-HT axons induced by MDMA.

Ex vivo experiments in rats confirmed the SPECT results obtained in monkey brain. Significant decreases in [123I]β-CIT accumulation were noticeable in all areas examined (except for the raphe nuclei) after treatment of rats with neurotoxic doses of MDMA. Reductions in rat brain in specific [123I]β-CIT binding ratios were highest in cortical brain regions, such as the occipital cortex (−74%), prefrontal cortex (−63%), parietal cortex (−65%), and temporal cortex (−55%). In subcortical brain regions, the highest reductions were observed in thalamus (−59%), midbrain (−57%), hypothalamus (−55%), and hippocampus (−53%). These observations are in agreement with previous in vitro and in vivo studies in which significant decreases in cortical as well as subcortical [125I]β-CIT binding were observed after treatment with MDMA as well as the 5-HT neurotoxin fenfluramine (Lew et al., 1996; Scheffel et al., 1992). In the fenfluramine study, decreases in [125I]β-CIT binding paralleled [3H]paroxetine reductions in the same animals (Scheffel et al., 1992).

The presence of SERTs in neocortical regions is well documented in human brain (Backström et al., 1989; Gurevich et al., 1996; Laruelle et al., 1988). Using the highly selective SERT ligand [3H]cyanoimipramine, the density of SERT in human brain tissue was estimated to be 107 fmol/mg protein in the cingulate gyrus of the prefrontal cortex, 82–97 fmol/mg in the temporal lobe, and 107 fmol/mg in the occipital lobe (Gurevich et al., 1996). However, there has been some discussion in the literature about the ability of [123I]β-CIT to bind to SERT in the cerebral cortex. Nevertheless, Farde et al. (1994) have shown that [11C]β-CIT uptake in monkey cortex could be displaced by 50% by citalopram, a selective SERT inhibitor, but not by GBR 12909, a selective dopamine transporter inhibitor. These findings are consistent with reports on binding of [123/125I]β-CIT to SERTs in the neocortex of rats (Boja et al., 1992; Scheffel et al., 1992). Unfortunately, we were not able to investigate cortical SERT reductions in the monkey brain with SPECT. Future studies should be conducted to validate the use of [123I]β-CIT SPECT in detecting SERT reduction in the cortex.

An interesting observation was that the density of SERT in the raphe nuclei in rat brain was unaffected by MDMA. This observation is consistent with previous autoradiography (Battaglia et al., 1991; Lew et al., 1996) and immunohistochemical (Hatzidimitriou et al., 1999; O'Hearn et al., 1988) studies with MDMA. These observations suggest that MDMA treatment does not cause loss of a particular group of 5-HT nerve cell bodies.

We observed increased (although not significantly, $P = 0.135$) striatal binding of [123I]β-CIT in MDMA-treated rats as compared to controls. Displacement studies have shown that striatal [123I]β-CIT uptake is associated with dopamine transporters, as it is displaced by GBR 12909 but not by citalopram (Laruelle et al., 1993). The inverse is true for the hypothalamic/midbrain region, suggesting that uptake in this region is associated with SERT. Previous studies have demonstrated increased binding of [123I]β-CIT to the DA transporter after administration of 5-HT reuptake inhibitors (Fujita et al., 1997; Scheffel et al., 1994). This probably reflects inhibition of [123I]β-CIT binding to the SERT and consequently increased availability to bind to dopamine transporters. Another possible explanation for this enhancement of binding is regulation of the DA transporter through inhibition of 5-HT uptake. It has been suggested that (rapid) regulation, such as posttranslational regulation, is evoked by the inhibition of 5-HT uptake (Fujita et al., 1997), for instance, by MDMA. However, the present observations made in rat striatum are in contrast to those observed in the rhesus monkey. Using [123I]β-CIT SPECT, we observed a 13% decrease in striatal binding 10 days after MDMA treatment and a reduction of 26% after 31 days. Whereas the present [3H]citalopram autoradiography data shows evidence of reduced striatal SERT after MDMA treatment in monkey, the SPECT data obtained in the same monkey suggest that, apart from SERT, dopamine transporter densities are also reduced after MDMA treatment. Since dopamine transporters greatly outnumber SERT densities in the striatum, binding in this region to SERT is relatively small compared to dopamine transporters and may therefore explain some, but not all, of the striatal reductions observed using SPECT. Therefore, the possibility that dopaminergic neurons are affected after MDMA treatment cannot be definitively excluded. Although previous studies have consistently failed to find any alterations in brain dopaminergic function following MDMA treatment, a recent study (Taffe et al., 2001) reported

significant reductions of the dopamine metabolite homovanillic acid in MDMA-treated rhesus monkeys when compared with a control group (but not pretreatment values). A possible explanation for the discrepancy between the observations in rat and monkey brain may be related to species differences, since it is well known that primates are more sensitive to MDMA than rats (Ricaurte et al., 1988; Slikker et al., 1988).

In the present study, the cerebellum was used as a reference region for nonspecific binding, enabling us to correct, among other things, for differences in tracer delivery between animals. Although there is good evidence that the cerebellum is innervated by 5-HT axons and that it therefore contains SERT, the density of SERT in the cerebellum is much lower than in other brain regions (Cortés et al., 1988; Laruelle et al., 1988; Fuxe et al., 1993; Bishop et al., 1993). Hence, while we cannot exclude the fact that MDMA-treatment may have affected "nonspecific" binding in the cerebellum, the commonly employed use of specific binding ratios, in which specific binding in the ROI is divided by cerebellar binding, would have resulted in an overestimation of SERT density and consequently an underestimation of MDMA's neurotoxic effects on 5-HT neurons. Additional studies are needed to investigate the presence of other brain regions devoid of SERT, better suited to serve as a reference region when calculating SERT with [^{123}I]β-CIT.

In summary, our data suggest that [^{123}I]β-CIT SPECT may be a valuable tool in detecting MDMA-induced 5-HT neurotoxic lesions in the hypothalamic/midbrain region. Future studies are required to further investigate the ability of [^{123}I]β-CIT to image SERT in the human cortex.

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