Effects of dose, sex, and long-term abstention from use on toxic effects of MDMA (ecstasy) on brain serotonin neurons

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Summary

Background 3,4-methylenedioxymethamphetamine (MDMA or ecstasy) is a popular recreational drug that has been shown to damage brain serotonin neurons in high doses. However, effects of moderate MDMA use on serotonin neurons have not been studied, and sex differences and the long-term effects of MDMA use on serotonin neurons have not been identified. We investigated the effects of moderate and heavy MDMA use, sex differences, and long-term effects of MDMA use on serotonin neurons in different brain regions.

Methods By means of flyers posted in "rave" venues in Amsterdam, the Netherlands, we recruited 15 moderate MDMA users, 23 heavy MDMA users, 16 ex-MDMA users who had stopped using MDMA for more than 1 year, and 15 controls who claimed never to have used MDMA. We studied the effects of MDMA on brain serotonin neurons using ^{123}iodine-2β-carbomethoxy-3β-(4-iodophenyl) tropane ([^{123}I]β-CIT)—a radioligand that binds with high affinity to serotonin transporters. Density of binding (expressed as a ratio of region-of-interest binding over binding in the cerebellum) was calculated by single-photon-emission computed tomography (SPECT).

Findings We saw significant effects of group and group by sex (p=0·041 and p=0·022, respectively) on overall [^{123}I]β-CIT binding ratios. In heavy MDMA users, significant decreases in overall binding ratios were seen in women (p<0·01) but not men (p=0·587). In female ex-MDMA users, overall densities of serotonin transporters were significantly higher than in heavy MDMA users (p=0·004), but not higher than in controls (p=0·524).

Interpretation Our results indicate that heavy use of MDMA is associated with neurotoxic effects on serotonin neurons, that women might be more susceptible than men, and that MDMA-induced neurotoxic changes in several brain regions of female ex-MDMA users are reversible.

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Introduction

3,4-methylenedioxymethamphetamine MDMA (ecstasy) is an amphetamine congener that has gained popularity as a recreational drug. Its perceived safety is at odds with evidence of MDMA neurotoxicity in animals: damage to serotonin neurons has been indicated by reductions in various markers unique to serotonin axons including brain serotonin, 5-hydroxyindoleacetic acid, and the density of serotonin transporters.[1–3] Since the serotonin transporter is located on the presynaptic terminal of the serotonin neuron, it is regarded as a reliable marker of neurotoxic changes. Recent positron emission tomography (PET) and single-photon-emission computed tomography (SPECT) studies have shown decreases in the number of central serotonin transporters in MDMA-treated primates and human MDMA users.[4–6]

These neuroimaging studies have revealed dose-related decreases in central serotonin neurons in heavy MDMA users. However, whether moderate use of MDMA can produce these changes,[7] and whether there is a level up to which MDMA has no observed adverse effect, is not known. Furthermore, differences in susceptibility to the neurotoxic effects of MDMA between the sexes have not been studied directly, although one study suggested a more pronounced subjective response to MDMA in women than in men.[8] Additionally, McCann and co-workers observed greater reductions in 5-hydroxy-indoleacetic acid in female than in male MDMA users,[9] suggesting that women are more susceptible than men to the neurotoxic effects of MDMA. Finally, although the short-term neurotoxic effects of MDMA on serotonin neurons have been studied extensively, little is known about the long-term effects on the human brain.[7] Studies in non-human primates have shown that up to 7 years after treatment with MDMA, some brain regions remain denervated while others show evidence of complete recovery.[10]

The fate of brain serotonin neurons after MDMA injury in the human brain remains to be established. This question is of interest since irreversible loss of serotonin neurons can result in the immediate or delayed onset of neuropsychiatric disorders in which serotonin has been implicated. Specifically, serotonin imbalance is thought to underlie depression, anxiety, panic disorder, and disorders of impulse control. In line with this association, many case reports document neuropsychiatric sequelae after MDMA use.[11]

The development of ^{123}iodine-2β-carbomethoxy-3β-(4-iodophenyl) tropane ([^{123}I]β-CIT)—a radioligand that binds with high affinity to dopamine and serotonin transporters—has permitted assessment of the density of serotonin transporters in the living human brain by means of SPECT.[12,13] β-CIT can adequately detect changes in the densities of cortical and subcortical serotonin transporters secondary to serotonin neurotoxicity.[14,15]

We investigated the effects of moderate and heavy MDMA use on the density of [^{123}I]β-CIT-labelled serotonin transporters, possible differences between men and women, and the effects of long-term abstention from MDMA use on [^{123}I]β-CIT-labelled serotonin transporters.

	Controls (n=15)		MDMA (n=15)		MDMA+ (n=23)		ex-MDMA (n=16)		p_{group}*	p_{gender}*
	Men (n=7)	Women (n=8)	Men (n=9)	Women (n=6)	Men (n=12)	Women (n=11)	Men (n=8)	Women (n=8)		
Demographics										
Age (years)	29·3 (6·9)	23·3 (1·3)	25·6 (7·5)	22·7 (2·8)	27·1 (6·0)	25·0 (4·1)	26·4 (6·2)	24·1 (4·7)	0·63	0·02
DART-IQ	104·7 (6·2)	106·9 (7·4)	111·2 (11·5)	112·2 (8·1)	106·0 (9·0)	104·5 (8·4)	105·9 (11·8)	102·0 (7·7)	0·10	0·73
Current depression	0	2	1	2	2	2	1	3	0·86†	
Alcohol and other recreational drug use										
Units of alcohol/week	14·1 (12·8)	7·1 (7·4)	18·2 (14·8)	5·3 (3·2)	13·0 (8·2)	5·8 (3·5)	4·5 (3·9)	7·9 (5·4)	0·14	0·00
Cigarettes/day	9·5 (3·3)	10·3 (6·1)	11·0 (6·5)	9·4 (9·2)	12·4 (13·0)	6·0 (7·1)	11·8 (8·5)	13·3 (8·6)	0·47	0·21
Cannabis joints in past 3 months	2·3 (0·6)	4·5 (5·0)	68·1 (6·5)	31·8 (51·6)	94·6 (153·0)	67·5 (101·9)	73·1 (110·4)	196·3 (369·3)	0·37	0·23
Amphetamine use in past 3 months	0	0	0·4 (0·8)	0	3·8 (7·4)	3·6 (5·5)	0	0	0·04	0·80
Usual dose amphetamine (g)	..	..	0·3 (0·2)	0·1 (0·1)	0·4 (0·3)	0·3 (0·3)	0·7 (0·4)	1·0 (0·8)	0·07	0·83
Cocaine use in past 3 months	0	0	1·2 (1·1)	0	4·2 (2·8)	4·4 (3·4)	0	0	0·09	0·74

Values are numbers or mean (SD). *Two-way analysis of variance. †χ^2 test. DART-IQ=score on Dutch adult reading test.

Table 1: **Demographics, prevalence of current depression, and exposure to other recreational drugs**

Methods

Participants

Participants were recruited by means of flyers distributed at venues associated with the "rave scene" in Amsterdam, Netherlands, with the help of UNITY—an agency that provides harm-reduction information and advice. Individuals selected were between 18 and 45 years, otherwise healthy, and had no psychiatric history.

Three different groups of ecstasy users were recruited: moderate users (MDMA group), heavy users (MDMA+ group), and ex-users (ex-MDMA group). The eligibility criterion for the MDMA group was previous use of a maximum of 50 tablets of ecstasy, whereas the MDMA+ group had to have used 50 tablets or more before the study. The ex-MDMA group had to have taken a minimum of 50 tablets but stopped using ecstasy for at least 1 year before the study. The cutoff point of 50 lifetime tablets was based on previous findings of increased risk of developing psychiatric disturbances in people with a lifetime consumption of 50 or more MDMA tablets.[16] Individuals who used drugs other than ecstacy were recruited from the same community source and were designated controls.

Participants agreed to abstain from use of all psychoactive drugs for at least 3 weeks before the study. After this abstention period, they were asked to undergo urine drug screening with an enzyme-multiplied immunoassay for amphetamines, barbiturates, benzodiazepine metabolites, cocaine metabolite, opiates, and marijuana before enrolment. Individuals underwent a fully structured computer-assisted diagnostic psychiatric interview (Composite International Diagnostic Interview, version 2.1) to screen for current axis I psychiatric diagnoses. After testing urine samples, exclusion criteria were: a positive drug screen, pregnancy, a severe medical or neuropsychiatric illness that precluded informed consent, and use of medication with affinity for the serotonin transporter (eg, serotonin reuptake inhibitors) that could compete for [^{123}I]β-CIT binding. Furthermore, the Dutch version of national adult reading test[17] was administered to estimate verbal intelligence.

Written informed consent was obtained from all participants, and the institutional Medical Ethics Committee approved the study.

Imaging

[^{123}I]β-CIT was prepared by oxidative radioiododestannylation of its corresponding trimethylstannyl precursor (Research Biochemicals International, Natick, MA, USA) by the Radionuclide Center, Vrije Universiteit, Amsterdam, Netherlands. Radiolabelling and purification by high-performance liquid chromatography yielded the tracer at high radiochemical purity (>99·0%) and high specific activity (>185 MBq/nmol). Potassium iodide was used to block thyroid uptake of free radioactive iodide.

SPECT studies were done with a brain-dedicated SPECT system (Strichman Medical Equipment 810X, Medfield, MA, USA). This 12-detector single-slice scanner has a full width-at-half-maximum resolution of about 7·5 mm. Transaxial slices parallel to and upward in 5 mm steps from the orbitomeatal line to the vertex were acquired after positioning of the individual in the camera with a fixed light source oriented along the orbitomeatal line. Each image consisted of about 15 slices (in a 64×64 matrix) with a 3-min scanning time per slice. The energy window was set at 135–190 keV. Scanning started 4 h

	MDMA (n=15)			MDMA+ (n=23)			Ex-MDMA (n=16)		
	Mean (SD)	Difference between sexes* (95% CI)	p	Mean (SD)	Difference between sexes* (95% CI)	p	Mean (SD)	Difference between sexes* (95% CI)	p
Duration of use (years)	4·1 (2·6)	–1·2 (–4·2 to 1·7)	0·38	4·6 (2·1)	–1·8 (–4·1 to 0·5)	0·12	4·6 (2·6)	1·2 (–1·7 to 4·0)	0·39
Usual dose (tablets)	1·4 (0·5)	4·2 (–56·7 to 65·1)	0·89	2·2 (0·7)	–0·8 (–1·3 to –0·3)	0·00	2·1 (1·0)	0·2 (–0·9 to 1·2)	0·76
Lifetime dose (tablets)	28·6 (17·8)	–2·1 (–23·1 to 18·8)	0·83	530·0 (621·1)	–630·8 (–1102·6 to –159·0)	0·01	268·1 (614·3)	282·4 (–380·0 to 944·7)	0·38
Lifetime dose (tablets/weight in kg)	0·4 (0·2)	0·1 (–0·2 to 0·4)	0·66	6·4 (8·0)	–7·1 (–13·8 to –0·35)	0·06	1·8 (1·3)	–0·2 (–1·7 to 1·3)	0·78
Time since last tablet (months)	3·6 (5·9)	–1·6 (–8·5 to 5·3)	0·62	2·3 (2·4)	0·7 (–1·4 to 2·7)	0·52	29·0 (20·4)	–16·1 (–36·7 to 4·6)	0·12

*Value for women minus value for men.

Table 2: **Characteristics of MDMA use**

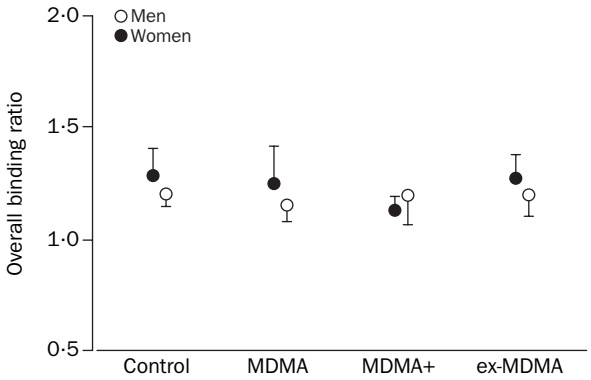

Figure 1: **Mean (SD) overall [^{123}I]β-CIT binding ratios (expressed as ROI binding over binding in the cerebellum) for different subgroups of MDMA users and controls**

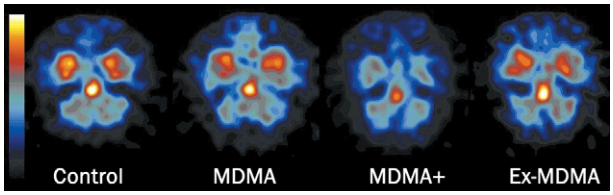

Figure 2: **[^{123}I]β-CIT SPECT images of a female control, a female moderate MDMA user (MDMA), a female heavy MDMA user (MDMA+) and a female ex-MDMA user (ex-MDMA)**
Transverse slices from brain at level of midbrain. In images from MDMA users, level of [^{123}I]β-CIT activity ranges from low (black) to high (white) and scaled to maximum in slice from control individual. Images show loss of serotonin transporters in midbrain of heavy MDMA user.

after intravenous injection of about 140 MBq [^{123}I]β-CIT—a time when specific binding to serotonin transporters is at a maximum.[18] Reconstruction and attenuation correction of all images were done as described previously.[19]

Density (mean counts per pixel) of the different regions of interest (ROIs) were calculated by an investigator unaware of the participant's history. We did the ROI analysis with a standard template constructed manually from coregistered magnetic resonance images in four control individuals. The template, including ROIs for the thalamus, frontal cortex, temporal cortex, parieto-occipital cortex, and occipital cortex was placed on three consecutive SPECT slices that showed best visualisation of the striatum (typically 30 mm above the orbitomeatal line). An additional template was constructed with an ROI for the midbrain and cerebellum. Binding in the cerebellum, which was assumed to be free from serotonin transporters, was used as a reference for background radioactivity (non-specific binding and free ligand). The ratios of [^{123}I]β-CIT binding were calculated by division of ROI binding by binding in the cerebellum.

Statistical analysis

Differences in continuous variables (log transformed if necessary) between the four groups were analysed by means of a two-way analysis of variance with group and sex as factors. Differences in the prevalence of depressed individuals between the four groups were investigated by χ^2 analysis.

To analyse the [^{123}I]β-CIT binding ratios at six different brain regions simultaneously, a mixed linear model was tested. By this technique, all data can be analysed simultaneously, taking into account within-individual and between-individual variations, and interaction effects and confounders, including direct estimates of the effect sizes, can be estimated. Using this analysis, we measured correlations (covariance structure) between binding ratios in different brain regions within the same individual. No mathematical pattern was imposed on the covariance structure within the same individual (unstructured).

Our basic model included brain region (six levels), group (four levels), sex (two levels), the interaction term between group and sex, and the interaction between group and brain region. We extended the model by including several potential confounders, including the presence of depression (two levels) and age (continuous), to determine whether a meaningful change in results occurred. From the final mixed model, we quantified the effect of MDMA on binding ratio in relevant groups or subgroups.

The relation between overall [^{123}I]β-CIT binding ratios and extent of previous MDMA use was assessed with Spearman's correlation coefficient. All mixed models were done in SAS version 6.12.

Results

There were no differences between the four groups with regard to age; verbal intelligence; and use of alcohol, tobacco, and cannabis. However, MDMA users reported more amphetamine and cocaine use than controls. The four groups did not differ in terms of the proportion of individuals with current depression (table 1). Men were on average 3·1 years (95% CI 0·6–5·6) older than women, and on average consumed 7·7 (3·7–11·8) more units of alcohol per week than women.

Apart from expected differences in amount of MDMA use between groups due to inclusion criteria, men in the MDMA+ group had used, on average, significantly more MDMA tablets than the women in this group (table 2). Additionally, the usual dose and total amount of MDMA tablets taken, expressed per kg bodyweight, was higher in the male MDMA+ subgroup than the female MDMA+ subgroup (table 2).

Overall mean [^{123}I]β-CIT binding ratios for the different groups are shown in figure 1. In the mixed linear model, the overall tests for group and group by sex were significant (p=0·041 and p=0·022, respectively), indicating a significant overall effect of MDMA use on binding ratios which was significantly different for men and women. Therefore, we have presented the differences between various groups for men and women separately.

Overall, [^{123}I]β-CIT binding ratios were significantly lower in female, but not male, heavy MDMA users than in controls (table 3), suggesting stronger effects of

	Men		Women	
	Mean (95% CI)	p	Mean (95% CI)	p
Control *vs* MDMA	0·040 (–0·061 to 0·142)	0·428	0·058 (–0·046 to 0·162)	0·269
Control *vs* MDMA+	0·025 (–0·067 to 0·117)	0·587	0·168 (0·078 to 0·259)	<0·01
MDMA *vs* MDMA+	–0·015 (–0·106 to 0·075)	0·737	0·110 (0·014 to 0·207)	0·026
Control *vs* ex-MDMA	–0·018 (–0·118 to 0·082)	0·723	0·031 (–0·067 to 0·129)	0·524
MDMA+ *vs* ex-MDMA	–0·04 (–0·132 to 0·046)	0·339	–0·137 (–0·227 to –0·046)	0·004

Table 3: **Difference in overall (^{123}I)β-CIT binding ratios**

Brain region	Men		Women	
	Mean (95% CI)	p	Mean (95% CI)	p
Midbrain				
Control *vs* MDMA	0·073 (–0·061 to 0·207)	0·280	0·091 (–0·045 to 0·227)	0·188
Control *vs* MDMA+	0·011 (–0·110 to 0·132)	0·857	0·154 (0·034 to 0·274)	0·013
Control *vs* ex-MDMA	–0·019 (–0·151 to 0·112)	0·767	0·030 –0·100 to 0·159)	0·649
MDMA+ *vs* ex-MDMA	–0·030 (–0·148 to 0·087)	0·607	–0·124 (–0·243 to –0·006)	0·040
Thalamus				
Control *vs* MDMA	0·026 (–0·198 to 0·249)	0·819	0·043 (–0·181 to 0·268)	0·702
Control *vs* MDMA+	0·078 (–0·122 to 0·278)	0·438	0·221 (0·022 to 0·421)	0·030
Control *vs* ex-MDMA	–0·095 (–0·312 to 0·121)	0·382	–0·046 (–0·262 to 0·170)	0·670
MDMA+ *vs* ex-MDMA	–0·174 (–0·369 to 0·022)	0·503	–0·268 (–0·464 to –0·071)	0·008
Frontal cortex				
Control *vs* MDMA	0·022 (–0·062 to 0·106)	0·607	0·039 (–0·048 to 0·126)	0·371
Control *vs* MDMA+	0·005 (–0·072 to 0·082)	0·901	0·148 (0·073 to 0·223)	<0·01
Control *vs* ex-MDMA	–0·001 (–0·085 to 0·082)	0·977	0·048 (–0·033 to 0·129)	0·241
MDMA+ *vs* ex-MDMA	–0·006 (–0·080 to 0·068)	0·872	–0·100 (–0·175 to –0·025)	0·010
Temporal cortex				
Control *vs* MDMA	–0·003 (–0·146 to 0·140)	0·972	0·015 (–0·130 to 0·160)	0·836
Control *vs* MDMA+	–0·009 (–0·138 to 0·120)	0·890	0·134 (0·006 to 0·262)	0·040
Control *vs* ex-MDMA	–0·051 (–0·191 to 0·089)	0·466	–0·002 (–0·140 to 0·136)	0·976
MDMA+ *vs* ex-MDMA	–0·042 (–0·168 to 0·083)	0·503	–0·136 (–0·262 to –0·010)	0·034
Parieto-occipital cortex				
Control *vs* MDMA	0·060 (–0·031 to 0·151)	0·120	0·078 (–0·016 to 0·171)	0·102
Control *vs* MDMA+	0·034 (–0·048 to 0·117)	0·411	0·177 (0·096 to 0·259)	<0·01
Control *vs* ex-MDMA	0·035 (–0·055 to 0·125)	0·439	0·084 (–0·003 to 0·172)	0·059
MDMA+ *vs* ex-MDMA	0·001 (–0·079 to 0·081)	0·985	–0·093 (–0·174 to –0·013)	0·024
Occipital cortex				
Control *vs* MDMA	0·065 (–0·030 to 0·159)	0·176	0·082 (–0·015 to 0·179)	0·096
Control *vs* MDMA+	0·032 (–0·054 to 0·118)	0·464	0·175 (0·090 to 0·260)	<0·01
Control *vs* ex-MDMA	0·025 (–0·068 to 0·118)	0·589	0·075 (–0·017 to 0·165)	0·107
MDMA+ *vs* ex-MDMA	–0·006 (0·077 to –0·089)	0·880	–0·100 (–0·184 to –0·016)	0·020

Table 4: **Differences in regional (^{123}I)β-CIT binding ratios in men and women**

MDMA exposure on serotonin neurons in women than in men. The overall effect of brain region was highly significant (p<0·01), with the correlations between the different brain regions ranging from 0·22 to 0·92. When we analysed the different brain regions separately (table 4), ^{123}I]β-CIT binding ratios were significantly lower in all brain regions studied in female, but not male, heavy MDMA users when compared with controls.

[123]I]β-CIT binding ratios were not significantly different from those of controls in male or female moderate MDMA users (table 3, figure 2), suggesting that moderate MDMA exposure has no effect on ^{123}I]β-CIT binding ratios.

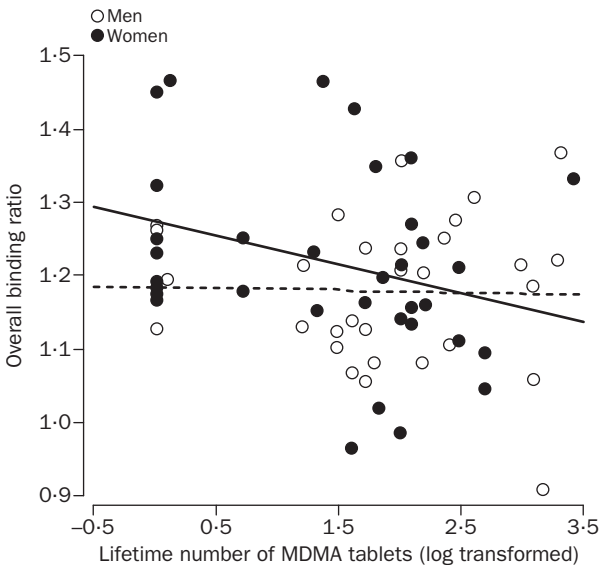

Figure 3: **Correlation between overall ^{123}I]β-CIT binding ratio of all brain regions studied and extent of previous MDMA use**

Overall, ^{123}I]β-CIT binding ratios were significantly higher in female ex-MDMA users than female MDMA+ users (p=0·004) but were not higher than those in controls (p=0·524, table 3, figure 2), suggesting that the effects of MDMA are reversible. Similar observations were made in the regional analysis (table 4). However, a trend towards decreased ^{123}I]β-CIT binding ratios in the parieto-occipital cortex (p=0·059) and the occipital cortex (p=0·107) of female ex-MDMA users was seen when compared with controls (table 4).

The effects of potential confounders were not significant (age p=0·492, current depression p=0·539). Inclusion of these potential confounders in the model did not change the overall effect of group on ^{123}I]β-CIT binding ratios.

A significant correlation was seen between overall serotonin transporter binding (mean binding ratios of all brain regions studied) and log-transformed extent of previous MDMA use in women but not men (women r=20·33, p=0·048; men r=0·07, p=0·699; figure 3).

Discussion

The results of our study suggest that MDMA use could lead to decreases in the density of brain serotonin transporters, and that men and women differ in their susceptibility to the neurotoxic effects of MDMA. We found that, in women, use of MDMA is associated with dose-related decreases in densities of brain serotonin transporters. Such a reduction was also seen in men, but this finding was not significant. The use of MDMA in quantities regarded as moderate does not lead to significant reductions in densities of serotonin transporters. Lastly, our data suggest that MDMA-induced decreases in serotonin transporters could be reversible in female MDMA users, but we cannot rule out the possibility that they might be long-lasting or only partly reversible in the parieto-occipital cortex and occipital cortex.

Decreases in densities of serotonin transporters are thought to be a consequence of MDMA-induced brain serotonin neurotoxicity, since similar reductions have been documented in rodents and primates with MDMA-induced serotonin injury.[1–3,5,10,20,21] We found that heavy MDMA use seems to be associated with global decreases in serotonin transporters. In MDMA-treated monkeys, the most severe serotonin depletion was found in the occipital cortex.[5] However, additional studies and converging lines of evidence are needed to delineate better the neurotoxic potential of moderate MDMA use in human beings.

In our study, women were more susceptible than men to MDMA dose-related decreases in [^{123}I]β-CIT-labelled serotonin transporters. Sex differences in the effects of MDMA exposure on radioligand binding to serotonin transporters have not been previously published. Semple and colleagues[6] only included male MDMA users in their study, and saw decreases in serotonin-transporter densities in posterior cortical areas. McCann and co-workers[4] found global decreases in the densities of these transporters when investigating male and female MDMA users. Contrary to the findings by Semple, we did not observe significant reductions in serotonin transporter densities in male MDMA users. Discrepancies between Semple's study and ours could be partly due to the fact that participants in our study abstained from psychoactive drugs (including MDMA) for at least 3 weeks, whereas they avoided such drugs for only 1 week in the study by Semple.

In our study, the observed sex difference might have been due to various things. First, inaccurate self-reporting of MDMA use might have biased the results. However, the positive correlation between reported MDMA exposure and densities of serotonin transporters in women suggests accurate self-reporting of MDMA use, so there seems to be no reason why men's self-reporting would not be accurate. Second, women, who on average have a lower bodyweight than men, might have been exposed to higher doses of MDMA on a mg/kg basis. However, on a tablet/kg basis, men had a higher exposure to MDMA than women (table 2). Third, sample size could have contributed to the apparent absence of neurotoxic effects in male MDMA users. However, a retrospective power analysis showed that, to detect with 80% power a significant reduction in overall [^{123}I]β-CIT binding in heavy MDMA users, seven women and 393 men were required in each group. Another potential explanation for the observed sex differences could be related to age. Age is known to affect serotonin-transporter densities,[18] so we analysed our results with age as a potential confounder. We did not see a significant effect of age on [^{123}I]β-CIT binding ratios, and addition of age to the model did not change the overall effect of group on specific to non-specific [^{123}I]β-CIT-labelled serotonin transporters. We therefore conclude that, after accounting for the differences in age, sex had an independent effect on [^{123}I]β-CIT binding ratios.

There is other evidence that the consequences and mechanisms of MDMA use are not identical in men and women. In line with our observations, McCann and co-workers observed greater reductions in 5 hydroxy-indoleacetic acid concentrations in the cerebrospinal fluid of female MDMA users than in male users.[9] Furthermore, Liechti and co-workers reported more pronounced subjective responses to MDMA in women than in men.[8] These observations support our finding that women could be more susceptible than men to the effects of MDMA; the consequences and mechanisms of action of other drugs are not identical in men and women.[22,23] The cause of these sex differences is unknown, but they could be related to differences in innate hormonal profiles,[24] volume and morphology of certain brain structures,[25] or monoaminergic neurotransmission. Future studies in larger experimental groups are needed to investigate further sex differences in the effects of MDMA exposure.

Our data also suggest reversibility of MDMA-induced changes in brain serotonin transporters in most of the brain regions of female MDMA users. A partly reversible neurotoxic action of MDMA on the human brain has been described by Gerra and co-workers,[26] when studying neuroendocrine responses after D-fenfluramine administration. Studies in primates show that MDMA-induced changes in serotonin terminal markers persist for longer than 7 years in the neocortex, particularly the pyriform and visual cortex, whereas brain regions proximal to the rostral raphe nuclei showed evidence of complete recovery. The distance of the serotonin terminal field to the rostral raphe nuclei influences recovery of serotonin axons after MDMA injury.[10] Clearly, to establish whether the presently observed changes in serotonin transporter densities in MDMA users are reversible, a prospective study design would be needed. However, since studies of MDMA in people are subject to ethical and methodological constraints, such a study would be difficult to do. Therefore, future studies in individuals with known MDMA-induced neurotoxicity need to be done to allow definite conclusions on reversibility or permanence of MDMA-induced changes in the human brain.

Decreased binding of the radioligand is assumed to reflect a decrease in the density of serotonin transporters and thus axonal loss, but several factors, such as allosteric changes in the binding unit of the protein, could also result in decreased binding. Nevertheless, central serotonin concentrations are also reduced after MDMA treatment, so this possibility is unlikely to be important.[27] Furthermore, correlative anatomical studies indicate that loss of presynaptic serotonin transporters in MDMA-treated animals is directly related to damage of serotonin axons and axon terminals.[20]

Several potential limitations of the current study should be mentioned. First, given the affinity of [^{123}I]β-CIT for both serotonin and dopamine transporters, our findings cannot be definitively ascribed to changes in the densities of serotonin transporters: the midbrain, thalamus, and cortex also contain dopamine transporters. However, displacement studies in animals have shown that binding of β-CIT is predominantly associated with serotonin transporters in these brain regions.[14] Furthermore, since we did not see differences in striatal [^{123}I]β-CIT binding ratios (obtained 24 h after injection of the radiotracer) between heavy MDMA users and controls, we conclude that the findings probably reflect differences in densities of serotonin and not dopamine transporters. Second, as with all retrospective studies, there is a possibility that pre-existing differences between MDMA users and controls underlie differences in serotonin-transporter densities—eg, people with low densities might be predisposed to use MDMA. Future studies taking the recently described functional polymorphism in the promoter for the serotonin transporter gene into account,[28] could be of interest. Third, since [^{123}I]β-CIT binding ratios are reduced in drug-free patients with depression,[29] we cannot rule out that the present findings are related to this disease. However, we saw no significant effects of current depression on [^{123}I]β-CIT binding, exclusion of depressed individuals from our statistical analysis did not affect the main outcomes of the present study (data not shown), and addition of current depression to the model did not change the overall effect of group on [^{123}I]β-CIT binding. Consequently, we conclude that the observed reduction in serotonin transporter densities is unlikely to be due to this potential confounding

variable. Fourth, observed decreases in the density of brain [^{123}I]β-CIT-labelled serotonin transporters are unlikely to be due to direct pharmacological effects of MDMA or other drugs, since MDMA-using participants reported that they had abstained from use of MDMA or other psychoactive drugs for at least 3 weeks before the study. Urine was screened to detect concealed recent MDMA use, but other than self-report, we were not able to ensure long-term abstention from MDMA. However, a recent survey in the Netherlands investigated the validity of the drug-history questionnaire that was used in this study. In 93% of the cases (n=594), the reported use of MDMA was in agreement with the drug-urine test.[30] In future studies, analysis of hair samples would be a useful way to ascertain previous use of MDMA.

Theoretically, the presently observed changes in [^{123}I]β-CIT binding to serotonin transporters could be due to drugs other than MDMA, since MDMA users in our study had more experience with other recreational drugs (mainly amphetamine and cocaine) than controls. However, since none of these drugs is a known serotonin neurotoxin in human beings, our findings are unlikely to be attributed to substances other than MDMA. An important part of the present study is that we minimised the potential confounding effects of psychosocial differences and use of other drugs between users and controls by studying participants from the same subculture. This situation differs from those of most previous studies, in which controls came from a university or general population.

In summary, our data indicate that heavy use of MDMA could be associated with neurotoxic effects on serotonin neurons in serotonin-rich brain regions. Our results indicate that women could be more susceptible than men to the neurotoxic effects of MDMA, and that MDMA-induced neurotoxic changes in brain regions of female ex-MDMA users might be reversible.

Contributors

L Reneman was the principal author of the paper, did the SPECT studies with [^{123}I]β-CIT, and analysed SPECT data. J Booij contributed substantially to the study design and the preparation of the paper. K de Bruin assisted with the execution of the SPECT studies, and contributed to the preparation of the paper. H Reitsma participated in the data analysis, statistical analysis, and manuscript preparation. F A de Wolff contributed to the neuropharmacological and toxicological aspects of the study design, and contributed to the preparation of the paper. W B Gunning contributed to the study design and preparation of the paper. G J den Heeten was the principal investigator and contributed to the preparation of the paper. W van den Brink was responsible for the psychiatric assessments, assisted with the statistical analysis, and contributed substantially to the preparation of the paper.

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