Investigating the potential neurotoxicity of ecstasy (MDMA). An imaging approach

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Chapter 11

Summary and general discussion
Summary

Findings in animals suggest that the popular recreational drug Ecstasy (3,4-methylenedioxymethylamphetamine or MDMA) might damage brain serotonin (5-HT) neurons in human beings. MDMA-induced 5-HT neurotoxicity has been demonstrated in animals at doses that approach or overlap those used recreationally by human beings, using a variety of experimental techniques. The effects of MDMA seem to be highly selective, exclusively damaging brain 5-HT neurons. If MDMA does produce 5-HT neurotoxicity in humans, there would be important ramifications for the mental health and psychological function of people who use this drug, because irreversible loss of 5-HT neurons may be responsible for an immediate or delayed onset of neuropsychiatric disorders in which 5-HT has been implicated. Specifically, 5-HT imbalance has been postulated to underlie psychiatric disorders including depression, anxiety, panic disorder, and disorders of impulse control. However, few data are available on the potential 5-HT neurotoxicity of MDMA in the human brain. Moreover, little is known on the long-term effects and potential functional consequences of MDMA-induced neurotoxicity. The recent development of brain imaging techniques such single photon emission computed tomography (SPECT) and magnetic resonance (MR) imaging have the potential to play an important role in our understanding of MDMA's short- and long-term effects in the human brain.

The aim of this thesis was to investigate the potential neurotoxicity of MDMA in the human brain with the help of different neuroimaging tools.

Part I: Introduction

In part I of this thesis, a general introduction and outline of the thesis was given.

Part II: Biological markers of neuronal loss

Part II investigated whether MDMA use is associated with neuronal loss. In Chapters 2 and 3 the effects of MDMA use on the serotonergic system were discussed, in Chapter 4 its effects on non-specific neurons, whereas Chapter 5 focuses on the dopaminergic system.

Chapters 2.1-2.3 investigated the radioligand best suited to study loss of serotonin (5-HT) neurons using SPECT by labeling of 5-HT transporters, a biological marker for the integrity of 5-HT neurons. It was concluded that [\(^{125}\)I]p-CIT is the radioligand best suited, among the \(^{125}\)I-CIT analogues developed so far to visualize 5-HT transporter densities. Next, the ability of [\(^{11} \)C]beta-CIT to detect MDMA-induced reductions in 5-HT transporter densities was determined in rat brain using ex vivo [\(^{11} \)C]beta-CIT binding assays and in monkey brain using [\(^{11} \)C]beta-CIT SPECT. It was concluded that [\(^{11} \)C]beta-CIT SPECT can reliably detect changes in 5-HT transporter densities secondary to MDMA-induced neurotoxicity in the hypothalamic/midbrain region, and possibly other 5-HT brain regions.

Having validated the use of [\(^{11} \)C]beta-CIT SPECT in detecting MDMA-induced 5-HT neurotoxicity, the effects of moderate and heavy MDMA use on the density of the [\(^{11} \)C]beta-CIT labeled 5-HT transporters were investigated in human MDMA users. The results are described in chapter 3.1. In addition, possible differences between males and females in the effects of MDMA exposure, and the effects of long-term abstinence from MDMA use on [\(^{11} \)C]beta-CIT labeled 5-HT transporters were analyzed. In female, but not in male, heavy MDMA users significant decreases in 5-HT transporter densities were observed, suggestive of MDMA-neuronal loss. MDMA-induced neurotoxic changes seemed to be reversible in most, but not all, brain regions of female ex-MDMA users. Finally, a trend was observed in female subjects suggesting that moderate MDMA use is associated with reductions in 5-HT transporter densities in the parieto-occipital cortex and occipital cortex, brain regions that seem to be particularly sensitive to MDMA's effects. Differences in 5-HT transporter densities between healthy male and female subjects were described in chapter 3.2. 5-HT transporter densities in the midbrain and DA transporter densities in the striatum were found to be significantly higher in females than in males. This study indicates the importance of taking gender into account in studies investigating the 5-HT and DA system, and may in part explain the gender differences observed in the neurotoxic actions of MDMA, as discussed in chapter 3.1.

In chapter 4, the effects of MDMA on another biological marker of neuronal loss, NAA, were studied in male subjects with proton MR Spectroscopy (\(^{1}H\) MRS). The ratios of NAA/Cr and NAA/Cho were significantly reduced in the frontal cortex of these male MDMA users as compared to control subjects, which correlated with the degree of MDMA exposure.

In chapter 5 the effects of ecstasy and amphetamine on DA neurons in the human brain were studied with [\(^{11} \)C]beta-CIT SPECT. Striatal DA transporter densities, a biological marker for the integrity of DA neurons, were significantly lower in combined ecstasy and intentional amphetamine users as compared to sole ecstasy users. DA transporter densities in sole ecstasy users did not differ from control subjects, suggesting that amphetamine, but not MDMA, is associated with loss of DA
neurons. Furthermore, the effects of methylphenidate, an amphetamine derivative which seems to lack 5-HT and DA neurotoxic potential, were studied on 5-HT and DA neurons in more detail in chapter 5.2. It was observed that DA transporter densities were significantly reduced in rat frontal cortex and hippocampus 5 days after treatment with methylphenidate in combination with the selective 5-HT2 receptor agonist quipazine. Activation of 5-HT2 receptors by quipazine potentiated methylphenidate-induced release of DA, which may have resulted in a reactive down-regulation of DA transporters.

Part III: Potential functional consequences of MDMA-induced neuronal loss

Part III of this thesis investigated whether the use of MDMA is associated with impairments of functions in which 5-HT is thought to play an important role.

Since 5-HT has been shown to regulate post-synaptic 5-HT2 receptor densities, in chapter 6 the effects of MDMA on post-synaptic 5-HT2 receptor densities were discussed. 5-HT2 receptor densities in human MDMA users were studied with [123]I-R91150 SPECT. In parallel, ex vivo [123]I-R91150 binding assays in addition to HPLC analysis of 5-HT levels were obtained in MDMA-treated rats. In rats, a decrease followed by a time-dependent recovery of post-synaptic cortical 5-HT2A receptor densities was strongly and positively associated with the degree of 5-HT depletion. In recent MDMA users, post-synaptic 5-HT2A receptor densities were significantly lower in all cortical areas studied, while 5-HT2A receptor densities were significantly higher in the occipital cortex of ex-MDMA users. The combined results of this study suggest that the compensatory up-regulation of 5-HT2A receptors in the occipital cortex of ex-MDMA users may reflect low synaptic 5-HT levels, because of MDMA-induced neurotoxic lesions.

It is known that brain post-synaptic 5-HT2 receptors play a role in the regulation of brain microvasculature. In chapter 7.1 5-HT2 receptor densities in MDMA users were studied with [123]I-R91150 SPECT, and was cerebral blood vessel volume (rCBV), studied with perfusion MR imaging. Low cortical 5-HT2 receptor densities were significantly associated with low rCBV values (implicating vasoconstriction), and high cortical 5-HT2 receptor densities with high rCBV values (implicating vasodilatation) in the globus pallidus and occipital cortex of MDMA users. These observations suggest a relation between the serotonergic system and an altered regulation of the brain microvasculature in human MDMA users. In chapter 7.2 it is demonstrated, using perfusion and diffusion MR imaging, that former MDMA use is not only associated with increases in rCBV values in the globus pallidus of abxinet MDMA users (as discussed in chapter 7.1) but also with an increase in the diffusional motion of water (ADC value) in the globus pallidus, possibly reflecting MDMA-induced axonal loss.

In chapter 8 the effects of moderate and heavy MDMA use on cognitive function were assessed using classic neuropsychological testing. In addition, the effects of long-term abstinence from MDMA use on cognitive function were studied. Finally, because 5-HT transporters may play an important role in cognitive functioning, the effect of a polymorphism in the 5-HT transporter promoter gene region (5-HTTLPR) on cognitive function was investigated. In line with previous studies, impairments in memory function were observed only in heavy, but not in moderate, users of MDMA with relatively intact performance in reaction times and tasks of attention and executive functioning. Similar observations were made in individuals who stopped using MDMA more than 1 year ago. No evidence for a role of 5-HTTLPR genotype in MDMA (ab)use or cognitive performance was observed.

Part IV: Linking biological markers of neuronal loss with memory function

Part IV further investigated whether the functional impairments described in part III of this thesis are associated with neuronal loss.

A study was described in chapter 9.1 in which 5-HT transporter densities and verbal memory function were evaluated in recent and ex-MDMA users. In recent, but not in ex-MDMA users, cortical 5-HT transporter densities were significantly lower when compared to control subjects (see also chapter 3.1). Memory performance in both recent and ex-MDMA users was significantly reduced (see also chapter 8). In line with this, no association between memory performance and cortical 5-HT transporters was observed, suggesting that while the neurotoxic effects of MDMA on 5-HT neurons in the human cortex may be reversible, the effects of MDMA on memory function may be long lasting. Similarly, chapter 9.2 evaluated the relationship between verbal memory function and neuronal dysfunction in the MDMA users, by using 1H MRS which measures NAA/Cr ratios (see also chapter 4). In MDMA users, memory function was strongly associated with NAA/Cr in the prefrontal cortex, in which greater decrements in memory function predicted lower NAA/Cr levels -and by inference greater neuronal dysfunction- in the prefrontal cortex of MDMA.
users. Chapter 9.3 investigated whether alterations in cortical post-synaptic 5-HT₂A receptors assessed using $^{123}$I-R91150 SPECT (see also chapter 6), are related to memory disturbances in MDMA users. Cortical 5-HT₂A receptor binding correlated negatively with memory function in MDMA users, but not in control subjects. The findings suggest an altered 5-HT neuronal function with correlated memory impairment in abstinent MDMA users.

Part V: Summary and conclusion
In chapter 10 contributions of brain imaging studies on the potential neurotoxic effects of MDMA and functional consequences are reviewed. An overview is given of PET, SPECT and $^1$H MRS studies employed, most of which show evidence of neuronal injury in human MDMA users. In addition, different neuroimaging tools are discussed that have investigated potential functional consequences of MDMA-induced 5-HT neurotoxic lesions. In chapter 11 a summary of this thesis is given, and a conclusion and implementation.

General discussion
From the results of studies described in this thesis and the review of the literature presented in chapter 10, it is concluded that MDMA-induced 5-HT neuronal loss can be studied in some 5-HT rich brain regions using $^{13}$NH$_3$-CIT SPECT. Using this and other imaging techniques that enable detection of neuronal loss, evidence was obtained suggesting that human MDMA users are susceptible to MDMA-induced neuronal loss. Females were found to be more susceptible to MDMA's neurotoxic effects than males. The effects are dose related, and reversible in most brain regions. No evidence was obtained suggesting that MDMA affects the dopaminergic system in human MDMA users, in contrast to amphetamine. It was also observed that MDMA use is associated with several impairments of functions thought to involve 5-HT. Of these functions, memory impairment in MDMA users was associated with some biological markers of neuronal loss. However, it is important to note that the conclusions made in this thesis heavily depend upon results of previous experimental animal studies showing selective decreases in 5-HT neuronal markers in MDMA-treated animals with documented neurotoxic lesions. The present conclusions can be justified in conjunction with these experimental studies. However, some important limiting factors associated with the studies conducted in this thesis related to: (1) population under study, (2) verification of drug usage and, (3) techniques used.

(1) Population under study - Most of the limiting factors associated with the populations under study have already been discussed in the thesis. They mainly concern baseline differences between MDMA users and controls in vulnerability factors. These are problems that characterize most research in this area, since these studies have been retrospective and potentially vulnerable to selection bias and confounding (Curran et al., 2000). However, in most studies presented in this thesis measures were taken to reduce the effect of potential pre-existing differences between MDMA users and controls by recruitment of control subjects from the same population as the MDMA users and by statically controlling for potential confounding variables. This differs conspicuously from most previous studies, where controls came from a university or general population. However, other potential confounders may still be relevant, because MDMA use had already occurred at entrance of the study. Vulnerability factors may predispose some people to experience more toxic effects following MDMA use. For instance, in the literature it was reported that one fatality had a serum level of 1.26 mg/l, while another patient, whose serum level was 7.0 mg/l received supportive treatments only, and survived (Brown et al., 1987; Campkin et al., 1992). In keeping with this idea, it was observed in this thesis that females may be more susceptible to MDMA's neurotoxic effects than males. Although the etiology of this gender difference is unknown, it may relate to hormonal influences, because gonadal steroid hormones, particularly estrogen, have been shown to modulate some aspects of the function of the serotonergic system (McQueen et al., 1999). However, the observed gender differences may also relates to differences between males and females in 5-HT transporter densities (see also chapter 3.), and/or combined use of other drugs that antagonize MDMA induced hyperthermia (such as alcohol) (Malberg et al., 1998). Other factors that may modify MDMA's neurotoxic potential include: (a) dose and patterns of MDMA use (Boot et al., 2000); (b) age (McCann et al, 2000); (c) 5-HT transporter polymorphism (Lesch et al., 1996); (d) pre-existing psychiatric morbidity (Schifano et al., 2000); (e) circumstances during MDMA use (e.g., temperature, noise, crowdedness) (Malberg & Seiden 1998); (f) metabolism of MDMA (Ramamoorthy et al., 2001; Tucker et al., 1994). Future studies in larger experimental groups are needed to further investigate these vulnerability factors.

(2) Verification of drug usage - In the studies presented in this thesis, it was impossible to determine exactly what drug at what dose was taken. Analysis of tablets sold as
'ecstasy' has shown that these may contain MDMA at doses ranging from 40 to 150 mg. However, they may also contain other drugs, including 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxymethamphetamine, amphetamines, ketamine, LSD or a range of other chemicals and combinations of chemicals. However, in the Netherlands the chemical composition of an ecstasy tablet it is fairly well known at the time of the study, because of the Drugs Information and Monitoring System (DIMS), a unique project to chemically monitor the ecstasy market. Over de period 1998-2000 the content of MDMA in an ecstasy tablet was on average 98 mg (Planij et al., 2001). In the studies described in this thesis, one had to rely upon retrospective accounts of drug history using a drug-history questionnaire. A recent survey investigated the validity of the drug-history questionnaire that was used in this thesis. It was found that in 93% of the cases the reported use of ecstasy was in agreement with the drug-urine test (Van de Wijngaart et al., 1997). In this thesis, drug usage and abstention period were further verified by urine drug screening. Blood and urine samples can detect drugs like cannabis 2-3 weeks after use, but MDMA and other amphetamine derivatives can be detected only 24-48 h after the last dose. Therefore, one can only objectively confirm abstention from cannabis but not MDMA in the 2-3 weeks before the study. However, since MDMA may compete with binding of \(^{18}F\)-CIT to the 5-HT transporter, it was important to perform urine screening to detect concealed recent MDMA use. In future studies, hair-sample analysis would be a useful way to assess more appropriately what drug was taken at what time and to ascertain previous use of MDMA.

(3) Techniques used - Although SPECT studies with \(^{11}C\)-CIT SPECT may be limited by several factors (eg, the low cortical uptake of \(^{11}C\)-CIT, its high affinity for both 5-HT and dopamine transporters, the use of a non-optimal reference region (cerebellum) and a relatively low spatial resolution) several studies in animals and non-human primates have shown that this technique can adequately detect MDMA-induced reductions in 5-HT transporter densities in several 5-HT rich brain regions. Since none of the currently available techniques is perfect, it is all the more important that converging lines of evidence are gathered, using a variety of techniques that point into the same direction. It is therefore noteworthy that data from the different studies presented in this thesis (SPECT studies of the 5-HT transporter; \(^{1}H\) MRS studies of NAA; SPECT evaluations of 5-HT2 receptor densities; perfusion and diffusion MRI; and cognitive studies) are all indicative of alterations of brain (5-HT) structure and function in MDMA users. Without doubt more optimal techniques to evaluate MDMA-induced neuronal loss will emerge in the future. For instance, more selective radioligands for the 5-HT transporter are being developed for SPECT that may be more sensitive in detecting MDMA-induced neuronal loss, such as for instance 5-iodo-2-[[2-2-[(dimethylamino)methyl]phenyl][thio]benzyl alcohol (\([\text{\textsc{II}}]\)DAM), which has high binding affinity and selectivity toward 5-HT transporters (Acton et al., 1999). However, these radioligands are currently not commercially available. Until then, future SPECT studies will need to investigate the sensitivity and specificity of \(^{11}C\)-CIT SPECT in detecting MDMA-induced neuronal loss. Furthermore, other techniques such as \(^{1}H\) MRS, perfusion and diffusion MR imaging may come to play an important role in the future, once validated in MDMA-treated animals. The combined use of these techniques provides additional insights into the neurotoxicity of MDMA in the human brain. For instance, co-registration of SPECT with MRI scans will help to resolve the relatively low spatial resolution of SPECT, combining functional with anatomical information. In addition, although in most studies a region-of-interest (ROI) type of analyses was performed, automatic voxel-based analysis may be more powerful than, but consistent with ROI analysis, and turn out to be a valuable tool in detecting small differences between MDMA users and controls.

There was a discrepancy between the different neuroimaging techniques used as to which brain regions were affected by MDMA. For instance, using \(^{11}C\)-CIT SPECT, reduced 5-HT transporter densities were observed in all (except for the thalamus) 5-HT rich brain regions studied of female MDMA users. However, using \(^{1}H\) MRS, reductions in NAA could only be demonstrated in frontal gray, but not occipital gray matter of male MDMA users. Using \(^{13}N\)O rCBV SPECT, long lasting alterations in 5-HT2 receptor densities were observed only in the occipital cortex, but not in other cortical brain regions studied in MDMA users. Finally, using perfusion and diffusion MR imaging, alterations in rCBV and ADC values were primarily observed in the globus pallidus of MDMA users. If every technique would measure the same biological marker, one would expect these different techniques to demonstrate MDMA-induced (5-HT) neuronal loss or loss of function equally, and in all 5-HT rich brain regions. However, since every technique studies a different aspect related to MDMA-induced neurotoxicity, this cannot be the case. Furthermore, every technique has its own sensitivity in detecting MDMA-induced neuronal damage. Therefore, future studies should be conducted to further validate these
Part V | Summary and conclusion

neuroimaging techniques in animals with known MDMA-induced neurotoxic lesions. In addition, more studies should be conducted combining neuroimaging studies with neuropsychological and psychiatric assessments to study links between localized brain damage and cognitive/clinical problems.

Implications

The results of the studies presented in this thesis are a (partial) confirmation of our hypotheses and therefore raise important questions as to the safety of MDMA by recreational users of this drug. However, before the findings of the present studies can be validly used in prevention messages and clinical decision making, some of the results will have to be (re)confirmed in secondary studies, particularly concerning gender differences and (ir)-reversibility of MDMA's neurotoxic effects. The findings presented in this thesis have answered some important questions regarding MDMA's effects on the human brain. Still, some crucial questions regarding the causality, course and clinical relevance of MDMA's neurotoxicity have not been answered. Since all studies have been retrospective, the results are potentially vulnerable to selection bias and confounding by factors that increase the probability of becoming an MDMA user. Therefore, at this moment, it cannot be ascertained that humans are susceptible to MDMA-induced 5-HT injury.

Clearly, only an experimental study can overcome these problems and ascertain that recreational MDMA use is neurotoxic in humans. However, given the existing data such a study is ethically not acceptable. One possible approach would be to perform longitudinal studies in high-risk groups in which subjects are compared in terms of brain pathology, cognitive function and clinical symptoms, before and after they took MDMA. These studies would not only confirm and better define the relationship between MDMA exposure and the development of neurotoxicity, but in addition determine whether individuals exposed to MDMA are at increased risk of developing neuropsychiatric dysfunction. Potential functional consequences of MDMA induced neurotoxic lesions are not yet clear but may include depression, anxiety, memory disturbance and other neuropsychiatric disorders in which 5-HT has been implicated (McCann et al., 2000; Parrott et al., 2000; Schifano et al., 2000).

Studies in humans can be strengthened considerably by studies in animals. Obviously, there is still a lot to know about MDMA and its neurotoxic effects, and future animal studies could be directed in a number of different directions. Currently, an aspect that has received considerable attention in the literature is, for example, the effect of a single MDMA dose (McCann et al., 2001). Future animal studies could be directed at better characterizing the effects of dosage schemes. Moreover, recent attention in the literature has been directed towards the potential harmful interaction between MDMA and other drugs. While an increasing number of MDMA users combine MDMA with 5-HT reuptake inhibitors (SSRIs) to dampen the dysphoria experienced after MDMA use (Boot et al., 2000), there are concerns that SSRIs potentiate acute toxic effects of MDMA (Hegadoren et al., 1999). In addition, the interaction between MDMA and other widely used illicit drugs should be identified in animal studies. They can also be directed to further validate the usefulness of imaging techniques in detecting MDMA-induced neurotoxic injury in the human brain and functional consequences thereof.

Neuroimaging techniques will greatly contribute to our understanding of MDMA's short- and long-term effects in the human brain. The fact that these techniques are non-invasive and most of them can be used repeatedly in the same subject is a very critical feature. However, it will not be possible to obtain a total picture of MDMA's neurotoxic potential and functional consequences in the human brain with neuroimaging alone. Only in conjunction with other fields, e.g. neuropsychology, psychiatry, neurochemistry and toxicology, more fundamental insights can be acquired, resulting in a better risk assessment of this potential pressing public health issue, and help to predict future demands on health care.

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