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### Neurotoxicity of ecstasy: causality, course, and clinical relevance

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Published: 01/01/2007

#### *Document Version*

Publisher's PDF, also known as Version of record

#### *Citation for published version (APA):*

de Win, M. M. L. (2007). *Neurotoxicity of ecstasy: causality, course, and clinical relevance*. [Phd-Thesis - Research and graduation internal, University of Amsterdam, Graduate School of Neurosciences].

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de Win, M.M.L.

**Publication date**

2007

**Document Version**

Final published version

[Link to publication](#)

**Citation for published version (APA):**

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# **Neurotoxicity of Ecstasy: Causality, Course, and Clinical Relevance**

Most of the studies described in this thesis were financially supported by a grant of The Netherlands Organization for Health Research and Development as part of their Addiction Program (ZonMw grant 310-00-036).

Printing of this thesis was financially supported by GE Healthcare Diagnostic Imaging, GE Healthcare Medical Diagnostics, Mallinckrodt Medical BV, and the BV Cyclotron VU.

Neurotoxicity of Ecstasy: Causality, Course, and Clinical Relevance  
Thesis, University of Amsterdam, The Netherlands

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Layout: Chris Bor, Medical Photography and Illustration,  
Academic Medical Center, The Netherlands  
Printed by: Buijten & Schipperheijn, Amsterdam, The Netherlands

ISBN: 978-90-9021492-4

# **Neurotoxicity of Ecstasy: Causality, Course, and Clinical Relevance**

**Academisch Proefschrift**

ter verkrijging van de graad van doctor  
aan de Universiteit van Amsterdam  
op gezag van de Rector Magnificus  
prof. dr. J.W. Zwemmer

ten overstaan van een door het college voor promoties ingestelde  
commissie, in het openbaar te verdedigen in de Aula der Universiteit

op  
vrijdag 2 maart 2007, te 12.00 uur

door

**Maartje Maria Léontien de Win**

geboren te Eindhoven

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Faculteit der Geneeskunde

*'Labor improbus omnia vincit'*

Voor mijn ouders

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**PART**

**I**

**Introduction**

CHAPTER

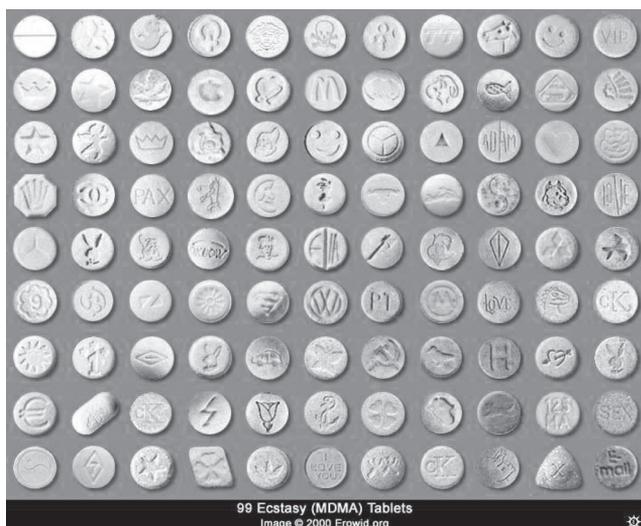
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# General Introduction and Outline



## GENERAL INTRODUCTION

Ecstasy, XTC, or E are popular names for the psychoactive drug 3,4-methylenedioxymethamphetamine, MDMA (see Figure 1)\*. MDMA is an entactogen, i.e., a psychotropic drug producing empathy, connectedness with others and increased



**Figure 1:** Example of the different appearances of ecstasy tablets.

suggestibility and in higher dosages acting as a stimulant and a psychedelic <sup>1</sup>. Users experience both an energizing effect, as well as distortions in time and perception, enhanced enjoyment from tactile experiences, a general sense of openness, empathy, euphoria, well-being and feeling of love.

### History of MDMA

MDMA was first synthesized in 1912 as a by-product when German pharmaceutical company Merck tried to synthesize Hydrastinin, a vasoconstrictive and hemostatic medicine <sup>2</sup>. It was patented but not further developed as a medicinal product. In the 1950s, MDMA was briefly researched by the U.S. Government as part of the warfare investigations, as a potential truth serum, but it proved to be unsuitable for this purpose. In 1965, the American biochemist Alexander Shulgin rediscovered ecstasy while searching for psychotherapeutic drugs. Especially in the mid 1970s, it was used as an adjunct to psychotherapy by psychiatrists and therapists who were familiar with the field of psychedelic psychotherapy <sup>3</sup>. In the early 1980s MDMA was introduced

\* In this thesis, the term 'MDMA' is used for ecstasy known to contain pure MDMA (laboratory conditions), the term 'ecstasy' is used for tablets/powder thought to be ecstasy although containment of merely MDMA was not confirmed (general practice).

as a non-medical drug under the name ecstasy. After MDMA was made illegal, in the US in 1985 and in the Netherlands in 1988, its popularity increased in the Netherlands and other Western countries. In the dance scene it became one of the most popular recreational drugs, because its properties gave the user energy to dance all night and feel overwhelmed with love from its surrounding in the same time. Since many years ecstasy is the most popular recreation drug in the Netherlands and most other Western countries besides cannabis (and of course alcohol). Since MDMA is illegal, ecstasy is produced in illegal laboratories, particularly in the Netherlands, Belgium and in Eastern Europe. As there is no regulation on its production, it is possible that tablets sold as ecstasy contain not only MDMA, but also other substances such as 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA), 3,4-methylenedioxyamphetamine (MDA), amphetamines, caffeine, paracetamol, or no psychoactive substance at all. However, between 2001 and 2004, the period that our study took place, purity of the ecstasy tablets in the Netherlands was very high. Results from pill-testing in this period confirm that in the Netherlands more than 95% of the tablets sold as ecstasy contained MDMA as the only (91.2%) or major (4.2%) component <sup>4,5</sup>. MDEA or MDA are major components in 1.5 % of the ecstasy tablets and only 1% of the ecstasy tablets contain amphetamine. The mean concentration of MDMA in an ecstasy tablet was 78 mg in 2003 and 83 mg in 2004 in the Netherlands. The price of an ecstasy pill has decreased since the 1980's and currently varies between 3 and 8 Euros <sup>6</sup>.

## Effects of MDMA on the brain

MDMA exerts its primary effects in the brain on neurons that produce the neurotransmitter serotonin (5-HT) to communicate with other neurons. MDMA stimulates secretion of large amounts of serotonin and inhibits the re-uptake of serotonin <sup>7</sup>. Serotonin plays an important role in regulating mood, aggression, sexual activity, sleep, and sensitivity to pain <sup>8</sup>. In addition, it has a smaller effect on dopamine and norepinephrine (noradrenaline) in the brain. This main effect on the serotonergic system is unlike other psychoactive drugs. Cannabis for example has its main effects on the endogenous cannabinoid system, amphetamine on the noradrenergic and dopaminergic systems and cocaine has its main effects on the dopamine system and to a lesser extent also on the noradrenergic and serotonergic systems.

## Neurotoxicity

The popularity of ecstasy as a recreational drug grew fast, probably encouraged as in the eighties and early nineties ecstasy was thought to be a relatively safe drug. Users did not get physically addicted to it and did not experience direct negative side effects. There were reports of users who died after ecstasy intake, mainly because of

overheating, aggregated by marathon dancing and high temperatures at overcrowded parties, but they were and still are regarded as rare incidents.

In 1985 the first studies in animals appeared and showed that MDMA was toxic to the serotonin neurons and destroyed their axons<sup>9</sup>. This was confirmed by numerous reports that showed that high or repeated-dose MDMA regimens can produce changes in indices of monoaminergic and axonal functioning in animals. This was indicated by reductions in brain markers of serotonergic axons, such as serotonin and its' most important metabolite 5-hydroxyindoleacetic acid (5-HIAA), reductions in densities of serotonin transporters, and the activity of tryptophan hydroxylase<sup>10-14</sup>. The magnitude of these changes varies with dose, species, and route of administration. Studies on rodents have shown that changes in the core temperature of animals can increase or decrease MDMA neurotoxicity. While some recovery does occur, a study in squirrel monkeys suggests that there may be permanent changes in axonal distribution<sup>15</sup>. Oxidative stress appears to play an important role in MDMA neurotoxicity, but the exact mechanisms are not exactly known. One of the leading theories is that the sustained acute pharmacological effects of MDMA may exhaust serotonergic energy sources leading to uptake of dopamine into the serotonergic cell and that the subsequent deamination of dopamine by monoamine oxidase-B (MAO-B) leads to the formation of the oxidizing hydrogen peroxide which may be a major contributor to the serotonergic toxicity<sup>16</sup>. Other theories are that MDMA itself or hepatic metabolites of MDMA are broken down into oxidizing metabolites, leading to damage<sup>17</sup>. Despite the extensive evidence from animal studies that MDMA is toxic to the axons of the serotonergic neuron, the risks of monoaminergic neurotoxicity in humans are still controversial and heavily debated<sup>18</sup>. Although animal studies enable researchers to overcome the limitation inherent to human studies, it is not possible to easily translate findings in animals to the human situation<sup>19</sup>. The dose / kg body weight used in animal studies are much higher than recreationally used by humans, because the rate of elimination of drugs varies between animals and humans. A technique of interspecies scaling can be used to predict drug elimination in various species<sup>20</sup>. However, this scaling method does not take into account metabolites and the relevance of active metabolite formation, which are important in MDMA-induced neurotoxicity but varies between species<sup>21</sup>. Moreover, higher core temperature increases the risk for neurotoxicity but because of physiological differences thermoregulation varies between species.

On the other hand, numerous studies in human ecstasy users have reported neuropsychobiological problems and neuroimaging studies have shown differences in the brain between ecstasy users and non-users. Indirect studies showed functional deficits in neurocognitive test performance, mainly in verbal memory, altered cognitive-emotional information processing, increased psychiatric symptoms, disordered sleep, sexual dysfunction, altered EEG patterns, reduced immuno-competence, and increased oxidative stress<sup>22</sup>. Imaging studies mainly reported decreased serotonin

transporter (SERT) densities in heavy ecstasy users, although there are indications that these effects are at least partially reversible<sup>23</sup>.

Ecstasy is the only known recreational drug with a specific toxic effect to the serotonergic system. In (meth)amphetamine users neurotoxicity was reported to the dopaminergic system<sup>24,25</sup>, whereas the adverse effects of cocaine seem to be mainly related to acute complications, decreased brain perfusion, and neuropsychiatric impairments without a clear toxic effect to specific neurons of the brain<sup>26</sup>. For cannabis there is little evidence for a long-term neurotoxic effect<sup>27</sup>.

Ecstasy studies, related articles in the mass media and information sites on the Internet about the potential dangerous effects of ecstasy on the brain may have contributed to the fact that ecstasy lost its 'harmless' image in the last 5 years, and a recent study from the US indicated that 73% of the ecstasy users consider ecstasy carry at least 'some risk'<sup>28</sup>. This might have led to the somewhat decreasing popularity of recreational ecstasy use in the recent years, also in the Netherlands<sup>6,29</sup>. Besides a more critical attitude towards ecstasy, these studies describe a certain ecstasy-tiredness among users because the novelty has disappeared, which translates into a renewed interest in the use of cocaine.

On the other hand, there is increasing interest in the use of MDMA as adjuvant in psychotherapy to reduce fear and to stimulate openness and suggestibility. This has led in 2001 to permission from the United States Food and Drug Administration (FDA) for testing MDMA in patients with post-traumatic stress disorder in conjunction with psychotherapy<sup>30</sup>.

Despite indications of ecstasy-induced neurotoxicity in humans, many questions are still unanswered, mainly related to the causality between ecstasy use and observed differences between ecstasy users and non-users. Therefore, the main aim of this thesis was to gain more insight in the effects of ecstasy on the brain, especially regarding causality, course and clinical relevance while considering the most important potential confounders.

## OUTLINE OF THE THESIS

In this thesis, consisting of five parts, we present our studies on the imaging techniques used to assess the effects of ecstasy on the brain and subsequently on psychopathological symptoms and neuropsychological performance. Part I gives a general overview and describes the aims, design and hypotheses of this thesis. Part II is about the use and validity of specific imaging techniques to assess potential neurotoxic effects of ecstasy. In part III the retrospective studies, mainly in heavy ecstasy users, are described. Part IV reports on the prospective studies in mainly

low-dose ecstasy users. Finally, in part V a summary, general discussion and the main conclusions are provided.

Most of the studies regarding the effects of ecstasy on the brain in this study were performed as part of the Netherlands XTC Toxicity (NeXT) study. The NeXT study was designed as a multidisciplinary study to overcome some of the methodological problems of previous studies. It uses a combination of cross-sectional and longitudinal (retrospective and prospective) approaches with a cross-sectional substudy among heavy ecstasy users and controls with variation in drug use, a prospective cohort substudy in ecstasy-naïve subjects with high risk for future ecstasy use, and a retrospective cohort substudy in lifetime ecstasy users and matched controls of an existing epidemiological sample. In these three substudies various neuroimaging techniques, neuropsychological tests and psychopathologic examinations are combined to study the causality, course, and clinical relevance of potential ecstasy-related neurotoxicity in humans. This thesis mainly focuses on the imaging and psychopathology findings in the retrospective substudy in heavy ecstasy users and in the prospective cohort substudy of the NeXT study.

## Part I: Introduction

**Chapter 1** gives a general introduction on the history, effects and potential neurotoxicity of ecstasy. **Chapter 2** describes the current state of knowledge about the potential neurotoxicity of ecstasy and the research questions that remained unsolved. It also gives a detailed outline of the objectives and methods of the Netherlands XTC Toxicity (NeXT) study that focuses on the causality, course, and clinical relevance of ecstasy neurotoxicity. The three substudies of the NeXT study and the different imaging, neuropsychological and psychiatric assessments that were used are described.

## Part II: Use and validity of imaging techniques in ecstasy research

Less than 10 years ago, it was not possible to directly study the effects of ecstasy in the living human brain, but since the development of different neuroimaging techniques an increasing amount of studies have been performed to evaluate the potentially toxic effects of ecstasy on the brain. In **chapter 3** imaging studies using single photon emission computed tomography (SPECT), positron emission tomography (PET) and proton magnetic resonance spectroscopy ( $^1\text{H}$ -MRS) are reviewed. Technical aspects, conceptual issues and future prospects of neuroimaging studies in ecstasy users are discussed.

One of the most frequently used imaging techniques to assess the serotonergic system in ecstasy users is [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT. [ $^{123}\text{I}$ ] $\beta$ -CIT is a radiotracer that binds to the terminals of the serotonergic axons, the SERT. In **chapter 4** the validity of [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT is assessed in detecting MDMA-induced neurotoxicity *in vivo* in rats. It compares *in vivo* and *ex vivo* measurements of [ $^{123}\text{I}$ ] $\beta$ -CIT uptake in the rat brain before and after administration of neurotoxic doses of MDMA. *In vivo* [ $^{123}\text{I}$ ] $\beta$ -CIT uptake was measured with a newly developed high-resolution pinhole SPECT scanner that makes it possible to perform SPECT studies in living small animals. **Chapter 5** investigates the validity of [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT to measure SERT densities in the living human brain. Although [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT has already been used to assess SERT densities in the human brain, there was still discussion about its validity because [ $^{123}\text{I}$ ] $\beta$ -CIT does not bind selectively to SERTs but also to dopamine transporters (DATs). In a double-blind, placebo-controlled, crossover design we compared [ $^{123}\text{I}$ ] $\beta$ -CIT uptake with and without blocking of the SERT using the selective serotonin reuptake inhibitor (SSRI) citalopram, both in SERT-rich and SERT-low brain areas.

Recently, the new radiotracer [ $^{123}\text{I}$ ]ADAM has been developed for selective imaging of SERTs with SPECT. The advantage of [ $^{123}\text{I}$ ]ADAM over [ $^{123}\text{I}$ ] $\beta$ -CIT is that it has a high affinity for SERTs but not for other transporters like DATs, which makes it possible to assess SERTs more selectively. Because no optimal protocol was available for clinical studies with [ $^{123}\text{I}$ ]ADAM SPECT, we examined the optimal time course of [ $^{123}\text{I}$ ]ADAM binding to central SERTs in humans. In **chapter 6** the results of this study are described.

### Part III: Retrospective studies in heavy ecstasy users

Both [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT and the advanced MR techniques  $^1\text{H}$ -MR spectroscopy ( $^1\text{H}$ -MRS), diffusion tensor imaging (DTI), and perfusion weighted imaging (PWI) were used to assess the effects of ecstasy on the brain in heavy ecstasy users in a retrospective substudy of the NeXT study, described in **chapter 7**. We aimed to assess specific/independent effects of ecstasy and relative contributions the other recreational drugs amphetamine, cocaine, and cannabis on the brain in a stratified sample with variation in type and amount of drugs that were used, because there is ongoing discussion whether previously reported neurotoxic effects are caused by ecstasy, by other drugs or by the combination of drugs.

Serotonin is important for many neurocognitive and psychopathological processes, like memory and mood, so it is important not only to look at sustained changes in neuroimaging parameters, but also at potential clinical consequences of ecstasy use. In **chapter 8** we assessed the effects of ecstasy use on mood and focussed on its association with serotonergic neurotoxicity, dose, and gender in humans. We measured mood with the composite international diagnostic interview (CIDI) and the Beck

depression inventory (BDI) and compared these measurements between groups of moderate ecstasy users, heavy ecstasy users, former heavy ecstasy users and drug-using, but ecstasy-naive controls. We also assessed whether mood scales correlated with SERT densities measured with [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT. In **chapter 9**, the effects of moderate, heavy and former ecstasy use on cognitive function were investigated. As females may be more vulnerable to the effects of ecstasy than males and as serotonin transporters are important in the regulation of synaptic serotonin transmission, we examined whether the effects of ecstasy on cognition were different for females and males and for subjects with a different polymorphism in the serotonin transporter promoter gene region (5-HTTLPR).

## Part IV: Prospective studies in low-dose ecstasy users

Previous studies suggested neurotoxic effects of heavy ecstasy use including increased depression, impulsivity and sensation seeking in ecstasy users, but it could not be excluded that observed differences between users and non-users were the cause and not the consequence of ecstasy use. Moreover it is unknown what the effects are of a low dose of ecstasy use on the brain. Part IV shows the results of the prospective substudy of the NeXT study, in which for the first time sustained effects of ecstasy were prospectively assessed in novel users. For this purpose a group of 188 ecstasy-naive subjects with an increased risk for future ecstasy use were assessed at baseline and followed for about 18 months. In **chapter 10**, the first 30 incident ecstasy users were assessed with a combination of advanced MR techniques and self-report questionnaires on psychopathology before and quite soon after their first ecstasy use (mean of 1.8 ecstasy tablets). At the end of the follow-up period we assessed the effects of ecstasy use on both SPECT and MR imaging parameters. **Chapter 11** compares these imaging parameters between all 59 incident ecstasy users with a mean use of 6.0 tablets and 56 persistent ecstasy-naive controls, corrected for their baseline measurements. In the same prospective group, the relationship between ecstasy use and self-reported depression, impulsivity, and sensation seeking was assessed, which is described in **chapter 12**. First, we assessed whether subjects with higher depression, impulsivity, and sensation seeking levels had a higher chance to start using ecstasy during the follow-up period. Then, we assessed whether depression, impulsivity, and sensation seeking had changed after first ecstasy use.

## Part V: Summary, general discussion and conclusions

Part V summarizes the most important findings of the studies presented in this thesis, discusses these findings in relation to the main research questions, and gives the main conclusions in English (**chapter 13**) and in Dutch (**chapter 14**).

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CHAPTER

2

# **The Netherlands XTC Toxicity (NeXT) Study: Objectives and Methods of a Study Investigating Causality, Course, and Clinical Relevance**

International Journal of Methods in Psychiatric Research 2005; 14: 167-185

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# ABSTRACT

## Background

This paper describes the objectives and methods of the Netherlands XTC Toxicity (NeXT) study focussing on the causality, course, and clinical relevance of ecstasy neurotoxicity. Previous studies suggest that ecstasy (3,4-methylenedioxymethamphetamine, MDMA, XTC) is toxic toward brain serotonin axons, but most of these studies have serious methodological limitations.

## Objectives and Methods

The current study is a combination of different approaches with three substudies: (1) a cross-sectional substudy among heavy ecstasy users and controls with variation in drug use, which will provide information about potential neurotoxic consequences of ecstasy in relation to other drugs, (2) a prospective cohort substudy in ecstasy-naïve subjects with high risk for future ecstasy use, which will provide information on the causality and short-term course of ecstasy use and potential neurotoxicity, and (3) a retrospective cohort substudy in lifetime ecstasy users and matched controls of an existing epidemiological sample, which will provide information on long-term course and outcome of ecstasy use in the general population. Neurotoxicity is studied using (a) different imaging techniques ( $\beta$ -CIT SPECT,  $^1\text{H}$ -MRSpectroscopy, Diffusion Tensor Imaging, Perfusion Weighted Imaging and functional MRI), and (b) neuropsychological and psychiatric assessments of memory, depression, and personality.

## Conclusions

The combined results will lead to conclusions that can be used in prevention messages, clinical decision-making, and the development of an (inter)national ecstasy policy.

## INTRODUCTION

Ecstasy (3,4-methylenedioxyamphetamine, MDMA \*, XTC) was introduced as a recreational drug in the early 1980s. In the early 1990s a steep increase in ecstasy use occurred when the substance became popular as a dance and party drug in many European countries. In the last few years, both incidence and prevalence of ecstasy use have stabilized. According to the most recent national general population survey among Dutch residents of 12 years and older in 2001, annual incidence was 0.5%, lifetime prevalence was 2.9%, and last month prevalence was 0.5% with higher prevalence among residents of Amsterdam (8.7% and 1.1%, respectively). Among a population of 16 million inhabitants this means that in The Netherlands approximately 70,000 people are monthly users of ecstasy. Nationally, lifetime and last month prevalence were highest among young adults between 20 and 24 years old (13.6% and 2.5%, respectively) <sup>1</sup>. A national school survey among Dutch students aged 12-18 indicated a peak in lifetime prevalence of ecstasy use in the second half of the 1990s, followed by a decrease with a lifetime prevalence of 2.9% and last month prevalence of 1.2% in 2003 <sup>2</sup>. Surveys among clubbers and ravers in Amsterdam also indicated that ecstasy use is over its peak; lifetime prevalence was 50.0% in 1995, 65.6% in 1998, and 52.7% in 2003; last month prevalence was 32.9%, 41.3%, and 19.4%, respectively <sup>3</sup>. Prevalence rates were higher among males than females in the general population <sup>1</sup>, as well as among students <sup>2</sup>. In spite of the widespread use of ecstasy among young adults, ecstasy use does not seem to constitute an addiction problem: on a yearly basis only 250-300 ecstasy users (0.4% of 70,000) seek advice or help for their ecstasy use at the addiction consultation and treatment centres in The Netherlands <sup>4</sup>.

On the other hand, there is increasing evidence from animal <sup>5,6</sup> and human <sup>7-11</sup> studies that the use of ecstasy might be toxic to serotonin axons in the brain. Serotonin is important for many physiological and neuropsychological processes, such as vasoconstriction, thermoregulation, memory, and learning <sup>12,13</sup>, so this could potentially lead to serious functional sequelae <sup>10,14-16</sup>.

Despite the vastly growing scientific literature on the effects of ecstasy on the human brain some crucial questions regarding the causality, course, and clinical relevance of the potential neurotoxicity of ecstasy have not been answered yet, mainly because of methodological limitations of most studies. These limitations include inadequate sampling of subjects and controls, small samples, lack of drug-use analysis, restricted dose ranges, short follow up periods, and the use of cross-sectional and retrospective designs with lack of baseline data and inadequate control of potential confounders <sup>17-19</sup>. Especially the use of other substances, such as amphetamines, cocaine, cannabis, alcohol,

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\* In this paper, the term 'MDMA' is used for ecstasy known to contain pure MDMA (laboratory conditions), the term 'ecstasy' is used for tablets/powder thought to be ecstasy although containment of merely MDMA was not confirmed (general practice).

and tobacco, could be major confounders in almost all existing ecstasy studies, because most ecstasy users are poly-drug users. Other important potential confounders are gender, age, lifestyle, serotonin transporter genotype, pre-existing psychiatric morbidity and pre-existing cognitive dysfunctioning.

First, the lack of baseline data leads to interpretative difficulties concerning the causality between ecstasy use and potential toxicity. Because of ethical and legal issues, most research on ecstasy-induced neurotoxicity in humans has been performed with cross-sectional study designs including retrospective assessment of ecstasy use. This leaves the possibility that observed differences between ecstasy users and controls were pre-existent<sup>20-23</sup> or that results were biased by confounding variables such as poly-drug use, gender, and lifestyle. In some of the better studies, at least some measures were taken to reduce selection bias through the recruitment of control subjects from the same population as the ecstasy users<sup>9,10</sup>. However, pre-existing differences in serotonergic functioning are still relevant because some of the serotonin-related subject characteristics (e.g. sensation seeking, impulse-related disorders) could probably be considered as predisposing factors for ecstasy use.

A second important issue about the consequences of ecstasy use that has not been elucidated is the course and long-term outcome of the assumed ecstasy-induced neurotoxicity. Of importance for clinicians and policy makers is to know whether changes in the serotonergic system are temporary and thus reversible, or lasting and thus irreversible. In non-human primates, MDMA produces reductions in serotonergic axon terminal markers that last for months or even years after cessation of drug exposure<sup>5,24</sup>. However, few human studies are available on the long-term effects of ecstasy use and the results are inconsistent and therefore inconclusive. Two studies reported normal densities of serotonin transporters (SERTs) in former ecstasy users<sup>9,25</sup>, while other studies (even in the same study population) reported long-lasting effects on memory function and symptoms of depression in ecstasy users who had stopped ecstasy use for at least one year<sup>10,14,15</sup>.

Third, little is known about the clinical relevance of observed serotonergic changes in humans. If ecstasy does damage serotonergic axons in humans, what functional consequences could be expected? Functional abnormalities seen in ecstasy users include memory disturbance, depression, impulsivity, and other neuropsychiatric disorders in which brain serotonin has been implicated<sup>14,16,18,26-28</sup>. Therefore, it is not only important to study the effects of ecstasy on serotonergic axons, but also to study the potential clinical consequences related to damage of these axons. Furthermore, changes in cerebral perfusion and cerebrovasculature of ecstasy users have been described<sup>29-31</sup>. Moreover, besides damage to the serotonin axons, several case reports have linked ecstasy use with the onset of Parkinsonism in humans, suggesting potential damaging effects of ecstasy on the dopamine system<sup>32-34</sup>, although the currently available evidence for dopaminergic damage is not convincing<sup>35</sup>. In addition, tablets

sold as ‘ecstasy’ may contain substances that are toxic to neuronal systems other than the serotonin system (such as the dopamine system). Some of the observed clinical consequences of ecstasy use, however, may not reflect long-term damage but only transient effects of the use of the drug. Therefore, studies comparing (long-term abstinent) former users and ecstasy-naïve controls on brain pathology, cognitive functioning, and clinical symptoms are of crucial importance to estimate its clinical relevance.

Finally, our understanding of dose-response characteristics and vulnerability factors, which may predispose some individuals to experience more negative effects following ecstasy use, is very limited. For example, it is important to find out whether brain pathology observed in heavy ecstasy users also occurs in less frequent users. Some researchers have argued that even a single moderate oral dose of MDMA might be neurotoxic in humans<sup>36,37</sup>, whereas others advocate the controlled use of MDMA as a therapeutic adjuvant for psychotherapy<sup>38</sup>. Furthermore, it has been suggested that time intervals between subsequent ecstasy exposures, environmental circumstances during ecstasy use (such as temperature, noise, dehydration, exhaustion, stress)<sup>39</sup>, and the combination with other substances (such as alcohol, cannabis, amphetamines)<sup>40-42</sup> could modify ecstasy-induced brain damage. Moreover, there are presumably important biological and psychobiological risk factors such as age, gender, neurotransmitter polymorphism, and pre-existing psychiatric morbidity that are related to individual differences in serotonergic functioning and to differences in vulnerability for the neurotoxic effects of ecstasy.

Because of limitations in current ecstasy research and the accompanying unanswered questions about its potential neurotoxicity, the Netherlands Research and Development Program on Substance Use and Addiction supplied a grant for the current Netherlands XTC Toxicity (NeXT) study addressing this important public health issue. The identification of specific health risks, such as cognitive impairment and brain damage, would provide a cogent argument for consumers to make informed decisions about recreational drug use. Ultimately, the NeXT study would help to predict future demands on healthcare. In the next paragraphs, the objectives and methods of this study are described and discussed.

## Objectives

The overall objective of the NeXT study is to come to better informed scientific knowledge regarding the neurotoxicity of ecstasy that can be used in prevention messages, clinical decision making, and the development of an (inter)national ecstasy policy.

Primary objectives are:

1. To study the causality of ecstasy use in observed brain pathology in humans;

2. To study the long-term course of brain pathology and related clinical characteristics in ecstasy users;
3. To study the clinical relevance of observed brain pathology in ecstasy users.

Secondary objectives are:

4. To study the dose-response characteristics of ecstasy use in the causation of brain pathology;
5. To study vulnerability and protective factors in the causation of brain pathology among ecstasy users;
6. To study potential neurotoxic consequences of ecstasy use in relation to the use of other drugs;
7. To study the presence of functional or structural damage to neurotransmitter systems other than serotonin following ecstasy exposure.

## DESIGN

### General design of the study

Only a long-term prospective study of serotonergic function in ecstasy-naive individuals randomly assigned to MDMA or placebo conditions could determine decisively whether recreational use is neurotoxic to human beings and whether these toxic effects are reversible or not. However, given the existing data on brain abnormalities in MDMA-treated animals and in human ecstasy users, such a study is ethically not acceptable. The NeXT study therefore studies causality, course, and outcome of various indicators of brain pathology (such as neuroimaging) and possible related clinically relevant symptoms (such as neurocognitive and psychiatric symptoms and disorders) of ecstasy neurotoxicity in a combination of three substudies. The outlines of the three substudies are summarized in the Figures 1, 2, and 3. The NeXT study includes

- A cross-sectional substudy of heavy ecstasy users and controls with variation in amount and type of drug use, which will provide information on potential neurotoxic consequences of ecstasy use in relation to the use of other drugs;
- A prospective cohort substudy in ecstasy-naive subjects with a high risk for future first ecstasy use, which will provide information on the causality and short-term course of ecstasy use and potential neurotoxicity, especially for low exposure levels; and
- A retrospective (historical) cohort substudy in lifetime ecstasy users and matched controls of an existing epidemiological sample, which will provide information on long-term course and outcome of ecstasy use in the general population and thus on potential public health consequences of ecstasy use in a Western society.

The combination of the three substudies with the use of similar assessment procedures in all substudies will provide important additional information regarding the neurotoxicity of ecstasy use in humans.

The total inclusion period for all three substudies was between April 2002 and June 2005 and final results are expected in the first half of 2006. All subjects had to be between 18 and 35 yrs of age. Exclusion criteria were: presence of a severe medical or neuropsychiatric disorder (for example, depression, psychosis, parkinsonism), use of psychotropic medications affecting the serotonin system such as selective serotonin reuptake inhibitors (SSRIs), pregnancy, intravenous drug use, and contraindications for MRI (such as claustrophobia or wearing a pacemaker). Subjects had to abstain from the use of psychoactive substances for at least two weeks prior to examinations and from alcohol for at least one week prior to examinations. Subjects were paid for their participation.

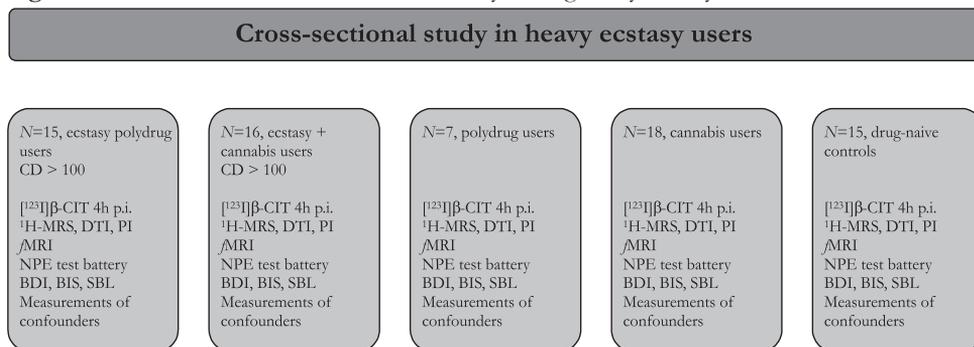
## Design and study samples of the substudies

### I. Cross-sectional substudy among heavy ecstasy users

The two main objectives of the cross-sectional substudy among heavy ecstasy users were

- To specify potential neurotoxic consequences of ecstasy use in relation to the use of other drugs; and
- To validate various imaging techniques for ecstasy research, especially  $^1\text{H-MR}$  spectroscopy, diffusion tensor imaging, perfusion weighted imaging, and functional MRI (see imaging parameters), which only have been used in a very few studies.

**Figure 1.** Outline of the cross-sectional substudy among heavy ecstasy users



The potential neurotoxicity of heavy ecstasy use was investigated with a retrospective assessment of drug use history and by comparing neuroimaging, neurocognitive, and psychopathological outcomes in a stratified sample of 71 subjects. Overall, subjects

can be classified according to five different profiles or 'groups' with variations in the amount and type of drug used:

1. A group of 15 heavy ecstasy polydrug users;
2. A group of 16 selective ecstasy and cannabis users;
3. A group of 7 polydrug controls with a history of heavy amphetamine and/or cocaine and cannabis use but very limited ecstasy use;
4. A group of 18 ecstasy-naïve cannabis users; and
5. A group of 15 drug-naïve controls.

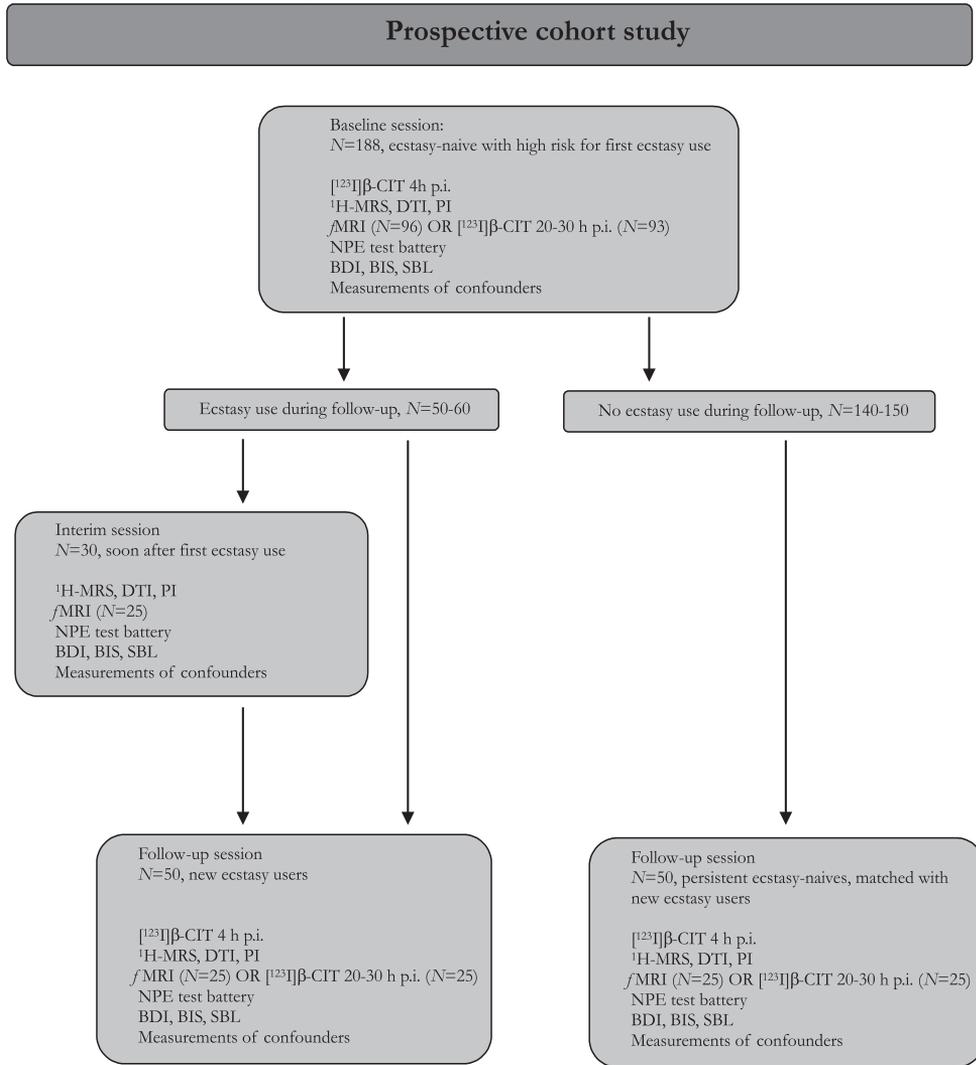
All subjects were included between October 2002 and January 2005. They were recruited through advertisements at dance- and drug-related sites on the Internet and in newspapers, through flyers at locations such as dance events, discotheques, youth fairs, universities, and colleges, and through word of mouth. Additional inclusion criteria for heavy ecstasy users (groups 1 and 2) were a cumulative dose (CD) of at least 100 ecstasy tablets and use of the last ecstasy tablet less than 6 months ago. The polydrug controls (group 3) had a history of regular use of amphetamines and/or cocaine, but a very limited use of ecstasy (maximum CD of 10 tablets). The ecstasy-naïve cannabis users (group 4) were matched to the heavy ecstasy users (groups 1 and 2) on gender, age, and CD of cannabis use. The drug-naïve controls (group 4) had never used psychoactive drugs, although they were allowed to have experience with the use of alcohol and/or tobacco just like the other groups. Part of the cannabis and drug-naïve controls (groups 4 and 5) were age-matched subjects taken from the baseline population of the prospective cohort study (see prospective cohort substudy).

In order to specify whether ecstasy users differ from non-users on indicators of neurotoxicity including clinical characteristics, outcome parameters of neurotoxicity will be compared between ecstasy users and ecstasy-naïve subjects. The comparisons will also indicate which imaging technique is most sensitive to detect neurotoxicity in ecstasy research. In addition, separate effects of various drugs (lifetime use of ecstasy, cannabis, amphetamine, and cocaine) on the outcome parameters will be assessed to examine whether drugs other than ecstasy contribute to the potential effect of ecstasy on indicators of neurotoxicity (see statistical paragraph).

## **II. Prospective cohort substudy**

To study the causal nature of ecstasy use on neuroimaging, neurocognitive, and clinical abnormalities observed in ecstasy users and to determine the effect of relatively low cumulative dosages of ecstasy, a sample of 188 ecstasy-naïve young adults (18-35 years) with a relatively high probability to start using ecstasy in near future was followed during a period of 12 to 24 months (Figure 2). They were actively recruited between March 2002 and April 2004, using a combination of targeted site sampling at locations, such as dance events, discotheques, youth fairs, universities, colleges, and parks; advertisement through a Website on the project and an Internet

Figure 2. Outline of the prospective cohort study



campaign; and snowball sampling referrals. Main criteria for inclusion were intent (probable or certain) to use ecstasy for the first time in the near future (3-5 points on a 5-points scale; 1 = certainly not; 2 = probably not; 3 = undecided; 4 = probably yes; 5 = certainly yes) and/or having one or more friends who already use ecstasy.

After baseline examination subjects had to complete questionnaires sent to them by mail about their drug use every three months during a follow-up period of one year. Besides assessing drug use through these questionnaires, the main outcome parameters were assessed up to three times: (T1) directly following recruitment, i.e. before first ecstasy use, in the total cohort (N = 188); (T2) soon after first ecstasy use

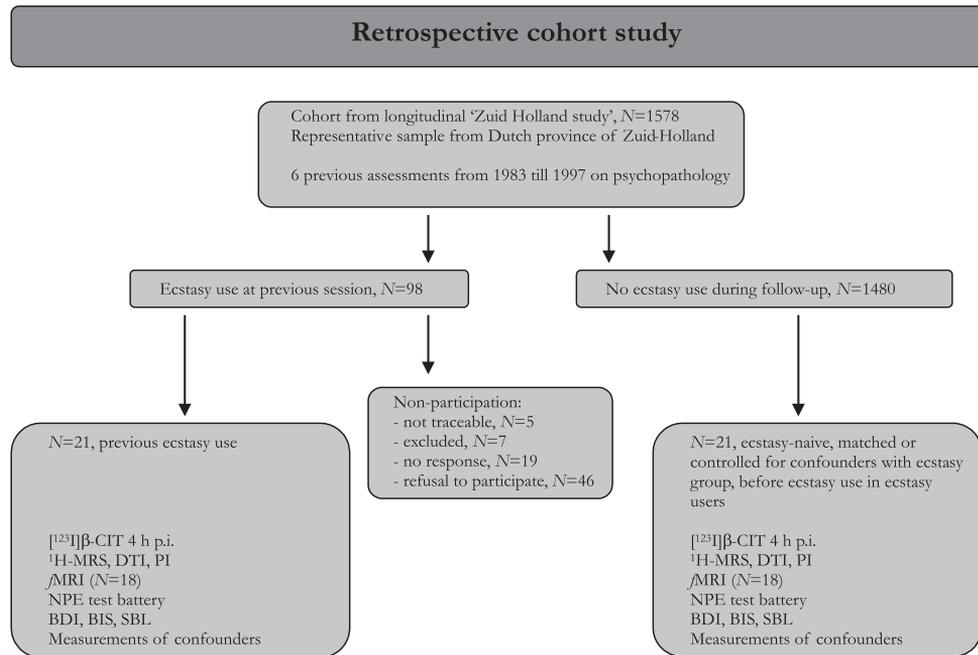
in the first 30 incident ecstasy users; (T3) between 12 and 24 months after baseline assessment in (T3a) all incident ecstasy users ( $N = 50-60$ ), and in (T3b) an individually matched (gender, age, DART-IQ, cannabis use) control group of persistent ecstasy-naive subjects ( $N = 50-60$ ). Single photon emission computed tomography (SPECT) imaging was only performed twice because of radiation exposure (at the first and third session). Follow-up measurements were finished in June 2005.

To study whether a low dose of ecstasy use is neurotoxic, outcome parameters of neurotoxicity will be compared between the first follow-up session soon after first ecstasy use in 30 incident cases (T2) and their baseline sessions before first ecstasy use (T1). We will also investigate whether ecstasy users differ from ecstasy-naive subjects on indicators of neurotoxicity and if so, whether differences were present before or developed after the first use of ecstasy. In order to examine this, indicators of neurotoxicity of incident ecstasy users (T3a) will be compared with persistent ecstasy-naive subjects (T3b) and both groups will be compared with their own baseline data (T1). Moreover, to assess whether certain variables (such as higher levels of depression, impulsivity and sensation seeking) can be considered as risk-factors for future ecstasy use in ecstasy-naive young adults, baseline data of incident ecstasy users (i.e. before first ecstasy use) will be compared with baseline data of persistent ecstasy-naive subjects. Finally, dopamine transporter (DAT) densities will be compared before and after ecstasy use in a subgroup of incident ecstasy users to specify possible effects of ecstasy use on the dopamine neurotransmitter system.

### III. Retrospective cohort substudy

To examine the potential public health consequences of ecstasy use in a Western society, a representative sample of lifetime ecstasy users and a matched control group of ecstasy-naive individuals were included in the retrospective (historical) cohort substudy. The participants of this substudy were selected from the longitudinal 'Zuid-Holland study' (Figure 3). This study started in 1983 with 2,600 subjects of Dutch nationality, aged 4 to 16 years (birth cohorts 1967 - 1979), randomly selected from the municipal registers from the Dutch province of Zuid-Holland, with both urbanized and rural areas. Of these, 2,076 (84%) participated in the first measurement in 1983<sup>43</sup>. Since then the sample was reassessed five times, most recently in 1997<sup>44</sup> when 1,578 subjects still participated (76.0% of the original sample of 2,076). Of these 1,578 subjects 98 indicated in 1997 during a psychiatric assessment with the Composite International Diagnostic Interview<sup>45</sup> that they had used ecstasy at least five times lifetime.

The group of lifetime ecstasy users and an individually matched control group of ecstasy-naive subjects were approached to participate in the current study. Outcome assessments in these groups have been started in May 2003 and finished in July 2005. The control group of ecstasy-naive subjects was matched for potential confounders

**Figure 3.** Outline of the retrospective cohort substudy

that were assessed prior to the first use of ecstasy. Matching variables included age, gender, use of cannabis, and internalizing (for example, anxiety, depression) or externalizing (for example, conduct disorder, ADHD) problems at age 4 to 16 measured with The Child Behavior Checklist (CBCL) <sup>46</sup>.

To assess whether lifetime ecstasy users of a representative sample differ on indicators of neurotoxicity, including clinical characteristics, from matched controls that never used ecstasy, outcome parameters will be compared between lifetime ecstasy users and non-users, while controlling for potential confounders. Moreover, correlations between characteristics of ecstasy use (such as lifetime CD, duration of abstinence) and outcome parameters will be analyzed in order to study the course and dose-response relationship of potential ecstasy-induced neurotoxicity.

## Assessments

### Exposure to ecstasy and other substances

Variables such as dose, dosing pattern, and circumstances under which ecstasy is used (such as temperature, noise, dehydration, exhaustion, stress) can influence the severity of neurotoxicity in animals <sup>47-49</sup>. Since this might also be true for humans <sup>39</sup>, we assessed these various aspects of ecstasy use with validated drug-use questionnaires <sup>50</sup>. The questionnaires were also used to assess use and frequency of use of other substances

such as cannabis, alcohol, tobacco, amphetamines, and cocaine. To exclude acute pharmacological effects of substance use on the main outcome parameters, subjects had to abstain from drug use for at least two weeks and from alcohol for at least one week prior to testing. This was checked through urine drug screening (enzyme-multiplied immunoassay for amphetamines, ecstasy, opiates, cocaine, benzodiazepines, cannabis, and alcohol). The absence or presence of prior ecstasy use and prior use of related substances such as amphetamines, MDA and MDEA will be checked in hair of all ecstasy users and of a random sample of 25% of the ecstasy-naive controls, using gas chromatography/mass spectroscopy analysis.

### **Outcome parameters (indicators of neurotoxicity)**

In the NeXT study indicators of neurotoxicity were assessed using a combination of neuroimaging, neurocognitive, and psychiatric assessments with techniques that already proved to be effective in detecting different aspects of serotonin-related neurotoxicity. In addition, currently known potential confounders (age, substance use, personality, depression, cognitive functioning, serotonin and dopamine transporter genotype) were assessed.

### **Imaging parameters**

Potential ecstasy-induced neurotoxicity can be studied *in vivo* in humans using various imaging techniques that assess different aspects of the structural and functional brain and provide complementary information. Most ecstasy-related imaging studies have been performed using positron emission tomography (PET) or SPECT with radiotracers that bind to serotonin transporters (SERTs) at the pre-synaptic terminal of the serotonergic axon. Because of radiation exposure these techniques are not suitable for multi-session follow-up studies. Multi-session follow-up studies are possible with magnetic resonance imaging (MRI) techniques. Moreover, the use of advanced MRI techniques makes it possible to study various other aspects of neuronal damage such as neuronal density and viability using proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS), axonal integrity using diffusion tensor imaging (DTI), and consequences for cerebrovasculature using perfusion weighted imaging (PWI). Furthermore, the neurophysiological correlates of cognitive brain functions such as working memory and attention can be studied using blood oxygen level dependent functional MRI (BOLD *f*MRI). As there is limited experience in ecstasy research with the various MRI techniques it is not known yet what indicators are most sensitive to detect ecstasy-induced serotonergic damage.

### ***SPECT***

Damage to the serotonergic axon can be studied in the living human brain by measuring the pre-synaptic SERT density. SERT is a structural element of the pre-

synaptic membrane and has been shown to be a reliable marker of MDMA-induced serotonergic neurotoxicity<sup>51</sup>. The radiotracer <sup>123</sup>Iodine-2β-carbomethoxy-3β(4-iodophenyl)tropane ([<sup>123</sup>I]β-CIT) that binds with high affinity to SERTs and dopamine transporters (DATs)<sup>52</sup> can be used in combination with SPECT to assess SERT (4 h after injection of the tracer) and DAT (20-30 h after injection of the tracer) densities<sup>53,54</sup>. Previous [<sup>123</sup>I]β-CIT SPECT studies have shown reduced SERTs in subjects with a history of ecstasy use<sup>9-11</sup>.

In the NeXT study [<sup>123</sup>I]β-CIT SPECT is performed 4 h post injection (p.i.) to measure SERT densities in subjects of all three substudies, once in the cross-sectional heavy user study and in the retrospective cohort study and twice (at baseline and at 12-24 months follow-up) in the prospective cohort study. SPECT scanning is also performed 20-30 h p.i. to assess DAT densities in a subgroup of subjects from the prospective cohort study who were not selected for fMRI assessment. For detailed description of the SPECT procedure the reader is referred to De Win et al.<sup>54</sup>.

### **MRI: <sup>1</sup>H-MRS, DTI and PWI**

<sup>1</sup>H-MRS, DTI, and PWI were performed in a single scanning session on a 1.5 Tesla MRI scanner.

*Single voxel <sup>1</sup>H-MRS:* <sup>1</sup>H-MRS allows studying of certain metabolites in the brain *in vivo*, such as N-acetylaspartate (NAA), choline-containing compounds (Cho), myo-inositol (mI) and creatine plus phosphocreatine (Cr). NAA exists almost exclusively within the neuronal cell bodies and axons and reductions in NAA are therefore associated with neuronal damage and impaired cognition<sup>55</sup>. Choline is increased in brain diseases that involve increased membrane breakdown, myelination or inflammation and is thought to reflect cellular density<sup>56</sup>. Myo-inositol is a putative glial cell marker<sup>55</sup>. The creatine peak is thought to be relatively constant between individuals and in most brain diseases<sup>57</sup> and therefore often used as an internal reference to calculate ratios. Previous studies in ecstasy users showed decreased NAA/Cr ratios in the frontal gray matter<sup>58</sup>, correlated to impaired memory performance<sup>59</sup>, and increased mI/Cr ratios in the parietal white matter<sup>60</sup>. However, the decreased NAA/Cr ratio was not confirmed by another recent study<sup>61</sup>.

In the NeXT study, single voxel <sup>1</sup>H-MRS was performed in three voxels of interest placed in left parietal white matter, in mid-frontal grey matter and in mid-occipital grey matter. Relative (using Cr as a reference) and absolute metabolite concentrations of NAA, Cho, and mI will be calculated.

*DTI and PWI:* With DTI it is possible to quantitatively measure diffusional motion of water molecules in the brain. In the normal situation this motion is restricted in amplitude and direction by cellular structures such as axons. Therefore the apparent diffusion coefficient (ADC) is lower and the fractional anisotropy (FA) is higher in the brain than in bulk water. Processes that disturb structural elements of the brain tissue

can result in increased ADC and decreased FA. Only one previous article reported preliminary findings of ADC measurements in ecstasy users, finding significantly increased ADC values in the globus pallidus of ecstasy users <sup>31</sup>.

Serotonin is involved in the regulation of brain microcirculation <sup>12</sup> and cerebrovascular accidents were described in ecstasy users <sup>62,63</sup> so it is of particular interest to study the cerebral microcirculation in ecstasy users, which is possible with PWI using the dynamic susceptibility contrast (DSC) technique. Previous studies already indicated that exposure to ecstasy may lead to cerebrovascular changes <sup>29-31</sup>.

### ***Functional MRI***

Functional MRI is a relatively novel imaging technique aimed at localizing and assessing cerebral functions, including memory and attention. Brain activity patterns that correspond with cognitive functions are obtained by contrasting experimental conditions with control conditions within the same session. Changes in performance and/or brain reactivity patterns on these tasks are expected to reflect the severity of ecstasy's neurotoxic effects. Cognitive domains of interest are selective/sustained attention, working memory, and long-term memory <sup>64,65</sup>. One of the important advantages of fMRI over behavioural measures of brain functioning is that fMRI can reveal abnormalities in the organization of brain networks, which may occur as an adaptive response to brain damage and which may be difficult to detect in behaviour. This added value of fMRI has been supported by some recently published papers on the neurotoxicity of ecstasy <sup>66,67</sup>, which reported neurophysiological changes in the brains of heavy ecstasy users while task performance was normal. However, the results have been inconsistent in showing ecstasy-related long-term neuronal effects in humans, as the same research group could not demonstrate statistically significant differences between ecstasy users and controls in two previous fMRI studies <sup>68,69</sup>.

In the NeXT study, fMRI is performed in all right-handed volunteers of the cross-sectional study among heavy ecstasy users, in all right-handed subjects of the retrospective cohort study, and in a subgroup (right-handed) of the subjects from the prospective cohort study (see Figure 2). Based on previous findings in neuropsychological literature the fMRI protocol was designed to focus on three cognitive domains, i.e. working memory, long-term memory, and selective attention. Verbal working memory was assessed using a modified Sternberg item-recognition task (see for details <sup>70</sup>). Long-term memory was investigated using a visual associative memory task, adapted from Henke et al. <sup>71</sup>. Thirdly, selective attention was measured using a visuo-auditory attention paradigm. All three tasks were presented in the scanner, and fMRI scans were acquired during performance of the tasks and during control tasks. With post-processing analysis of the fMRI scans, brain activity patterns were assessed for each subject. Use of control tasks avoids the potential confound of changes in basic brain perfusion.

## Neuropsychological and psychopathological parameters

As serotonin modulates many neuropsychological processes, it can be expected that ecstasy-induced damage to serotonin axons leads to impairment of functions in which serotonin is involved, such as impulsivity, mood disorders, and memory function. Previous research on the functional consequences of serotonergic neurotoxicity induced by ecstasy showed converging evidence of impairment in memory<sup>10,16,72</sup>. However, studies on the effect of ecstasy use on mood, impulsivity, and sensation seeking are less conclusive because there are indications that symptoms of increased depression, impulsivity, or sensation seeking might be pre-existing or even predispose subjects to ecstasy use<sup>14,23,73</sup>. They could be thought of as influencing memory deficits as well. In the NeXT study subjects were assessed on a battery of tests on various aspects of cognitive functioning and with self-report questionnaires on depression and personality traits.

### *Neuropsychological parameters*

The neuropsychological test battery in the NeXT study includes tests that have proven to be sensitive to ecstasy-related neurotoxicity and tests related to functions or brain areas that are thought to be affected by ecstasy use (for example, prefrontal cortex, occipital cortex, hippocampus). Moreover, tests were selected by their sensitivity to detect subtle impairments in younger persons. The following cognitive domains were tested: working memory, verbal memory, visual memory, visuospatial ability, and verbal intelligence.

- *Working memory/executive functioning*: Impaired function of working memory in ecstasy users was found in several studies<sup>64,74</sup>. The PASAT (Paced Auditory Serial Addition Test)<sup>75</sup> was administered in the current study to measure working memory and information processing accuracy. Subjects have to add numbers presented by a recorded male voice to a preceding number. A Dutch adaptation of the Digit Span (Wechsler D, subtest of Wechsler Adult Intelligence Scale- revised WAIS-R) was used to measure attention and working memory<sup>76</sup>. The version of Lindeboom gives a more reliable difference score between repeating digits in forward and in backward order by offering subjects one series of digits extra per length. Previous studies found decreased scores on the Digit Span in ecstasy users<sup>72,77</sup> while others did not<sup>78</sup>. Finally, we used the Iowa Gambling Task to measure decision-making and risk-taking behaviour<sup>79</sup>. It provides participants with choices from four decks of cards, each associated with a specific degree of reward or punishment.
- *Verbal Memory*: The most substantial evidence for cognitive deficits in ecstasy users is on impaired functioning of ecstasy users on verbal memory tasks<sup>10,16,80,81</sup>. Verbal memory can be measured using the Rey Auditory Verbal Learning Test (RAVLT; Rey, 1964 192 /id}. In the current study a Dutch version was administered<sup>82,83</sup>.

Subjects have to memorize a series of 15 words in five learning trials. Immediate recall is tested after each trial. Delayed recall and recognition are measured after 20 minutes.

- *Visual Memory*: Previous studies on non-verbal memory reported inconclusive results <sup>84,85</sup>. We used a computerized adaptation of the Memory for Designs test <sup>86</sup>. The original test with 14 figures was split in two separate tests to obtain a parallel version. After a slide show of seven figures, five seconds each, subjects have to draw the figures from memory. The show is repeated five times. Delayed reproduction is measured after 15 minutes.
- *Visuospatial functioning*: Also studies on visuospatial functioning produced contradictive results <sup>84,85</sup>, although there are indications that brain areas such as the parieto-occipital and occipital cortex, involved in visuospatial functioning, are affected by ecstasy use <sup>9</sup>. In the current study the first test to measure visuospatial functioning was the Mental Rotation Task <sup>87</sup>. Participants were presented with 20 pairs of block designs drawn from different points of view. Within 3 min they had to judge whether pairs of designs are identical or different. A computerized and adapted version of the Judgement of Line Orientation (JOLO) <sup>88</sup> was used to test visuospatial working memory. The JOLO requires subjects to identify which 2 of 11 lines presented in a semicircular array have the same orientation in two-dimensional space as two target lines. The target lines in our assessments were only shown for one second, directly followed by the 11 lines.
- *Verbal Intelligence*: The Dutch Adult Reading Test (DART), the Dutch version of the National Adult Reading Test <sup>89</sup>, was administered to estimate pre-morbid verbal intelligence (DART-IQ) as it is relatively insensitive to cognitive impairment caused by neurological disorders <sup>90</sup>.

### *Psychopathological parameters*

Current depression was assessed using the Beck Depression Inventory (BDI) <sup>91</sup>. The BDI is a 21-item self-report rating inventory which measures characteristic attitudes and symptoms of depression in the week prior to assessment. The BDI has proven to be a reliable and valid indicator of depression <sup>92,93</sup>. Increased BDI scores were reported in recent and former ecstasy users <sup>14,81</sup>.

Also, increased impulsivity scores were reported in ecstasy users <sup>26,94</sup>. The Dutch version of the Barratt Impulsiveness Scale (BIS-11) was used in the current study to assess impulsivity <sup>95</sup>. The Dutch BIS-11 contains 31 self-report items that have to be scored from 1 to 4. Total scores and subscale scores on attentional impulsivity ('difficulty in concentrating'), motor impulsivity ('acting without thinking'), and non-planning impulsivity ('thinking about the present rather than the future') will be calculated.

The ‘Spannings Behoefte Lijst’ (SBL), a Dutch adaptation of the Sensation Seeking Scale<sup>96</sup>, was used to measure sensation seeking<sup>97,98</sup>. The SBL contains 51 sensation-seeking items, for which respondents have to indicate on a five-point scale to what extent they (dis)agree with the statements. Both total scores and scores for subscales on thrill and adventure seeking (TAS), experience seeking (ES), boredom susceptibility (BS), and disinhibition (DIS) will be calculated. Increased sensation/novelty seeking in ecstasy users was reported in various studies<sup>22,99,100</sup>.

### Potential confounders

Various potential confounders that have been identified in literature were assessed in all subjects included in the substudies:

- *Substance use other than ecstasy*: Almost all ecstasy users use other substances as well, so the use and frequency of use of cannabis, alcohol, tobacco, amphetamines, cocaine, LSD, mushrooms, and other substances, were assessed using questionnaires described in the ‘exposure to ecstasy and other substances’ paragraph.
- *Demographic variables*: Demographic variables such as age, gender, level of education of subjects and their parents, ethnicity, and lifestyle were documented.
- *Gonadal hormones*: Detailed data regarding menstrual cycle and usage of oral contraceptives were obtained because it has been suggested that female ecstasy users are more vulnerable for subjective and neurotoxic effects of ecstasy<sup>7,9,101</sup>. Therefore, in all female subjects of the prospective and the retrospective cohort substudies who do not use oral contraceptives, 17- $\beta$ -oestradiol and progesterone were measured because these hormones modulate some functional aspects of the serotonergic system<sup>102</sup>.
- *Serotonin transporter genotype*: A genetic contribution to the expression of SERTs has been described, in which the activity of the long allele of the SERT promoter region has been shown to be twice that of the short allele<sup>103</sup>. The serotonergic polymorphism was assessed in the participants of the prospective and the retrospective cohort substudies to investigate the effect of pre-existing (genetic) differences in serotonergic function between ecstasy users and non-users.
- *Pre-existing psychiatric morbidity and cognitive dysfunctioning*: These potential confounders were assessed by the neuropsychological test battery and the psychiatric self-report questionnaires as described above.

## Statistics

### Power analysis

*SPECT, <sup>1</sup>H-MRS, DTI, PWI, and memory performance*: Previous SPECT findings<sup>9</sup> indicated that 8 females (effect size  $d = 0.16$ ) and 31 males ( $d = 0.08$ ), thus a group of 39 subjects per group, would provide ample power to demonstrate a difference in

SERT densities between pre-post assessments (prospective study) and between lifetime ecstasy users and ecstasy-naïve controls (retrospective studies), if such a difference exists. This power estimate is in general agreement with two other imaging studies conducted with ecstasy users, which showed that 25 subjects were needed per group to demonstrate a difference in serotonergic transporter densities using PET (males and females together) <sup>8</sup> and 32 using SPECT (only males) <sup>11</sup>. The sample sizes in all three substudies would also be big enough to detect effect sizes of 28%, 21%, and 31% on outcome parameters measured with <sup>1</sup>H-MRS, PWI, and memory performance (especially on RALVT), respectively, as indicated by previous studies <sup>10,31,58</sup>.

*fMRI*: Previous studies indicated that changes in cognitive abilities are small, but significant, after moderate ecstasy use. For reliable measurement of cognition-related functional brain activity patterns a sample size of about 10-12 subjects is required. To detect differences reliably between ecstasy users and controls, 10-12 subjects would be required per group. As brain activity patterns might differ between male and female ecstasy users, 20 to 24 subjects per group would be required in order to obtain representative samples for both genders.

### Statistical analyses

We hypothesized *a priori* that if ecstasy use is indeed neurotoxic, ecstasy users would differ on various imaging parameters (increase of ADC, rCBV, Cho, mI and decrease of [<sup>123</sup>I]β-CIT uptake ratios, FA, NAA), on BOLD fMRI parameters (increased activity or alterations in patterns of activation), as well as on parameters of neurocognitive functioning (such as decreased memory) and psychopathology (such as increased depression, impulsivity, sensation seeking) compared to non-users (cross-sectional substudy and retrospective cohort substudy) or compared to their own baseline values prior to first ecstasy use (prospective cohort substudy).

For the cross-sectional substudy among subjects with variations in amount and type of drugs used, parameters of neurotoxicity will be assessed using linear multiple regression analysis with lifetime use of ecstasy, cannabis, amphetamine, and cocaine as separate regressors. It is expected that this will provide information about the relative contributions of the various drugs on the main outcome parameters. The regression model will also control for factors other than drug use, such as gender, age, and DART-IQ.

For the prospective cohort substudy, follow-up data will be compared between incident ecstasy users and persistent ecstasy-naïve subjects using (multivariate) analysis of variance (ANOVA/MANOVA), including baseline measurements and significant confounders (such as age, gender, use of cannabis, amphetamines, and cocaine) as covariates (ANCOVA, MANCOVA). In order to prevent the loss of subjects due to incomplete data general linear mixed models could be applied in the analysis of the longitudinal data.

For the retrospective cohort study, parameters of neurotoxicity will be compared cross-sectionally between lifetime ecstasy users and matched non-users. An analysis of covariance will be used with main confounders (such as age, gender, cumulative dose of ecstasy, use of cannabis, internalizing and externalizing psychopathology at age 4-16 measured with the CBCL, prior to first ecstasy use in the group of lifetime ecstasy users) as covariates. Correlations between characteristics of ecstasy use (e.g. lifetime CD, duration of abstinence) and outcome parameters will be analyzed using a linear regression analysis.

### **Ethical considerations**

The NeXT study was approved by the local medical ethics committee. To rule out any suggestion that we approve or stimulate the use of ecstasy (especially in ecstasy-naïve subjects) volunteers were informed about potential negative consequences of ecstasy use. In addition, each participant had to sign a document giving informed consent, which states that participation was voluntary, that ecstasy is potential harmful and that the examiners do not have the intention to stimulate the use of ecstasy.

## **DISCUSSION AND CONCLUSIONS**

This article described the objectives, design, study populations, assessments, and statistical issues of the NeXT study with its focus on causality, course, and clinical relevance. To our knowledge this is the first large-scale ecstasy study using various imaging techniques and a combination of both cross-sectional and longitudinal (prospective and retrospective) approaches. It includes novel users with low CD as well as heavy users with high CD of ecstasy and adequate controls for confounders (partly measured prior to first ecstasy use).

The first substudy, including two groups of heavy ecstasy users (both polydrug users and selective ecstasy users), two comparison groups (polydrug users and cannabis users), and a drug-free control group, is especially designed to assess the potential neurotoxic consequences of heavy ecstasy use in relation to other drug use. Although some previous studies indicated that signs of neurotoxicity in ecstasy users might not be related to merely ecstasy use but rather to the use of different other psychoactive drugs<sup>40-42,104</sup>, only very few studies adequately controlled for use of other substances. The advance of the current study over previous studies is that we recruited a specific sample ( $N = 71$  in total) with specific variations in amount and type of drugs used in such a way that they are virtually uncorrelated, allowing for multiple regression analysis to tease out drug-specific effects while benefiting from the statistical power of a large total sample size. The results will give insight in the relative contributions of the different drugs on the cognitive impairments and serotonin-related neurotoxicity

found in heavy ecstasy users. In addition, this study will provide knowledge about the sensitivity and suitability of different imaging techniques, especially the MRI techniques such as <sup>1</sup>H-MRS, DTI, PWI, and BOLD fMRI, in studying the potential neurotoxicity of ecstasy. Most previous imaging studies used PET or SPECT with radiotracers that bind to SERTs at the terminal of the serotonin axon. However, the use of imaging techniques without radiation involved (for example <sup>1</sup>H-MRS, DTI, PWI, and BOLD fMRI) would make it possible to perform multi-session follow-up studies in future. Moreover, these techniques enable us to study different aspects of neuronal damage, complementary to the assessment of SERT densities as measured with PET or SPECT techniques. Despite the advantages of these techniques, few studies on the neurotoxicity of ecstasy using functional MRI <sup>66,68,69</sup>, DTI <sup>31</sup> or PWI <sup>30,31</sup> have been published to date and previous studies using <sup>1</sup>H-MRS were inconsistent <sup>58-61</sup>. The current study aims to fill this gap with an exploration of the specific opportunities and limitations of these new methods for ecstasy research.

The prospective substudy, using a naturalistic approach, will enable us to test the causal role of ecstasy use in serotonergic damage, to study a possible dose-response relationship, and to establish the short-term course and outcome of (various) indicators of brain pathology and possibly related clinical relevant symptoms after ecstasy use. To our knowledge, this is the first prospective study on ecstasy neurotoxicity comparing neuroimaging and neurocognitive assessments before and after first ecstasy use. It is therefore the most innovative substudy of the three and offers major methodological advantages over most previous studies. Assessment of main outcome parameters in both incident ecstasy users and persistent ecstasy-naïve subjects will enable us to control for several potential confounding effects (use of drugs other than ecstasy, personality, lifestyle, and so forth). The sampling technique of subjects with high risk for first time ecstasy use will lead to enough incident cases of first ecstasy use with generally low exposure levels and a few cases with higher levels of ecstasy exposure. The interval measurements relatively soon after the first ecstasy use in a subgroup of 30 subjects make it possible to study the effects of a single or low-dose of ecstasy on the brain. Although the issue of single or low-dose use of ecstasy and its effects on the brain has got relatively little attention in research until now, this issue is highly relevant. Only 20-30% of the ecstasy users use ecstasy on a regular basis (CD > 25 lifetime) <sup>105</sup>, while most ecstasy users do so on a low continuation rate and probably quit ecstasy use after a certain period of time. Moreover, there is a growing interest in the possible medical benefits of low dose ecstasy administration in certain groups of patients. Recently, the Food and Drug Administration (FDA) of the United States has approved two pilot studies using ecstasy as a therapeutic agent. South Carolina researchers study the effects of ecstasy in 20 patients suffering from post-traumatic stress disorder <sup>106</sup>. In addition, Harvard researchers will study whether ecstasy can help terminally ill cancer patients by reducing their fears, pain, and stress <sup>107</sup>. In this

context, the importance of our substudy on the effects of a single or low dose ecstasy use is evident. On the other hand, because of the sampling technique of subjects with high risk for first time ecstasy use, this group will not be representative for all ecstasy users. Moreover, given the relatively short follow-up period (maximum 24 months) this cohort will not provide data on long-term ecstasy abstainers and subsequently this cohort will not provide answers to the course and (long-term) outcome of neurotoxicity in ecstasy users.

To overcome these lacunae, the retrospective cohort substudy is performed with a representative sample of lifetime ecstasy users and a matched control group of ecstasy-naïve individuals. The most important advance over previous studies is that the research population is more representative for general ecstasy use in the Western society than most previous studies that mainly involved heavy ecstasy users. Therefore, this substudy will provide optimal data on the potential public health consequences of ecstasy use in a Western society. Moreover, because these subjects were involved in a longitudinal cohort study from childhood, we are able to retrieve potential confounders from available data that were acquired prior to first use of ecstasy. The group of lifetime ecstasy users in this cohort will predominantly consist of experimental and low level recreational users. Given the age range of this cohort and the low continuation rate of ecstasy use, the majority of the ecstasy users in this cohort will have stopped the use of ecstasy years before the current assessment. As a result, this cohort is very suitable to study the long-term course and outcome of the various indicators of brain pathology and possible symptoms related to ecstasy use in the general population.

The combination of the three substudies assessing different samples with the same combination of neuroimaging, neuropsychological, and psychopathological instruments to study various indicators of neurotoxicity, is needed to answer the research questions and obtain a comprehensive understanding of the use of ecstasy and its potential hazards. Because the same parameters are used in all three substudies this will improve the comparability of the different results which is essential for explaining and interpreting the results from the three substudies.

However, there will be some limitations involved. First, many potential confounders are involved in the effects of ecstasy on the brain. With the combined design of the three substudies we try to assess most of the known confounders, such as use of amphetamines, cocaine, cannabis, alcohol, and tobacco, baseline serotonergic functioning, gender, age, demographics, gonadal hormones, serotonergic transporter genotype, and pre-existing psychiatric morbidity and cognitive dysfunctioning. However, sample sizes of the three substudies are probably too small to correct adequately for all of these confounders simultaneously, especially in both retrospective substudies. Moreover, as the cumulative doses of ecstasy used in the prospective and retrospective cohort substudies will be relatively low, the potential effects on the brain

are probably smaller than in heavy users. Therefore, the samples sizes of 21 per group in the retrospective study, but even the relatively big sample sizes of about 50 per group in the prospective substudy might be relatively small to detect potential effects.

Inherent in the non-experimental approach is uncertainty about variances in dosage and purity of the ecstasy tablets taken by the subjects, although surveys in the Netherlands confirm that in 2002 95% of the tablets sold as ecstasy mainly contained MDMA or a related compound (MDA or MDEA) <sup>4</sup>. These percentages were even higher in 2003 and in 2004. There will also be some confounding introduced by biased sampling or polydrug interactions, although the designs of the substudies are aimed to control for confounders as good as possible. Even in the prospective substudy it is possible that the incident ecstasy users are more likely to use other substances at baseline and at follow-up than the persistent ecstasy-naïves, although both groups are from the same baseline group, recruited in the same way, and both with the intention to use ecstasy in near future. Moreover, the environmental circumstances under which ecstasy was taken and the simultaneous use of other substances will be heterogeneous. As it is not ethical to provide ecstasy tablets to humans in an experimental setting, there is still a need for separate animal studies to study some of the aspects of ecstasy neurotoxicity (such as vulnerability and protective factors, and the risk of neurotoxicity when ecstasy is used in combination with other substances) in a controlled setting.

## Conclusion

The NeXT study uses a combination of cross-sectional and longitudinal (retrospective and prospective) approaches and a combination of various imaging techniques, and neuropsychological and psychopathologic examinations to study the causality, course, and clinical relevance of potential ecstasy-related neurotoxicity in humans. The combined results on course and outcome of brain pathology and related symptomatology are expected to result in scientific knowledge that can be used in prevention messages, clinical decision making, and development of (inter)national ecstasy policy.

## ACKNOWLEDGEMENTS

The NeXT study was financially supported by a grant of The Netherlands Organisation for Health Research and Development as part of their Addiction Program. We thank the Addiction Research Institute of the University of Utrecht for the disposal of the questionnaires on drug use and Stanford University for the disposal of DTI sequences. We thank all researchers, physicists, biostatisticians, physicians, research

assistants, and research students at the Academic Medical Center Amsterdam, the University Medical Center Utrecht, the Bongers Institute, and the Erasmus Medical Center –Sophia Children’s Hospital, Rotterdam who contributed and still contribute to this project.

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**PART**

**III**

**Use and validity of imaging techniques  
in ecstasy research**

# CHAPTER 3

# **Neuroimaging Findings with MDMA/ Ecstasy: Technical Aspects, Conceptual Issues and Future Prospects**

Journal of Psychopharmacology 2006; 20: 164-175

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# ABSTRACT

## Background

Users of ecstasy (3,4-methylenedioxymethamphetamine; MDMA) may be at risk of developing ecstasy-induced injury to the serotonin (5-HT) system. Previously, there were no methods available for directly evaluating the neurotoxic effects of ecstasy in the living human brain. However, development of *in vivo* neuroimaging tools have begun to provide insights into the effects of ecstasy on the human brain.

## Aim

Single photon emission computed tomography (SPECT), positron emission computed tomography (PET) and proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) studies, which have evaluated ecstasy's neurotoxic potential will be reviewed and discussed in terms of technical aspects, conceptual issues and future prospects.

## Results

Although PET and SPECT may be limited by several factors such as the low cortical uptake and the use of a non-optimal reference region (cerebellum) the few studies conducted so far provide suggestive evidence that people who heavily use ecstasy are at risk of developing subcortical, and probably also cortical reductions in serotonin transporter (SERT) densities, a marker of serotonergic neurotoxicity. There seem to be dose-dependent and transient reductions in SERT for which females may be more vulnerable than males. <sup>1</sup>H-MRS appears to be a less sensitive technique for studying ecstasy's neurotoxic potential.

## Future Directions

Whether individuals with a relatively low ecstasy exposure also demonstrate loss of SERT needs to be determined. Because most studies have had a retrospective design, in which evidence is indirect and differs in the degree to which any causal links can be implied, longitudinal studies in human ecstasy users are needed to draw definite conclusions.

## INTRODUCTION

On the surface ecstasy (3,4-Methylenedioxymethamphetamine, MDMA) appears to be a safer drug than alcohol and cocaine, at least in the short term. However, a recent study indicated that 73% of ecstasy users view the drug as carrying at least ‘some risk’<sup>1</sup>. This may relate to evidence from animal studies indicating that MDMA produces toxic effects on brain serotonin (5-HT) axon terminals. The first studies on MDMA’s neurotoxic potential in animals were published in the early 1980s. In view of ecstasy’s popularity as a recreational drug, animal studies demonstrating serotonergic neurotoxicity after MDMA administration at doses that overlap with those used by humans, and the role serotonin plays in several essential functions such as mood, emotion, memory, sleep, pain, and higher order cognitive processes, it is important to determine whether ecstasy is neurotoxic to humans. In contrast to the numerous animal studies addressing MDMA’s neurotoxicity, the number of studies investigating the neurotoxic potential in humans is limited. This is probably because previously no methods were available to evaluate the neurotoxic potential directly. However, several attempts have been made to study the neurotoxic potential of ecstasy indirectly. For example, some studies have evaluated cerebrospinal fluid 5-HIAA, the major metabolite of serotonin, concentrations in ecstasy users and found either normal<sup>2</sup> or decreased levels<sup>3,4</sup>. Neuroendocrine challenge tests are another strategy for detecting serotonin dysfunction by indirect means<sup>5</sup>. However, recently *in vivo* neuroimaging tools, such as positron emission tomography (PET), single photon emission computed tomography (SPECT), and several magnetic resonance (MR) imaging applications have begun to directly provide insights into the effects of ecstasy on the living human brain. These imaging-techniques have identified a range of structural and functional consequences of ecstasy use and may be useful in the study of yet unknown but potential clinically relevant long-term effects. However, at present the findings in humans are not conclusive. Meanwhile, some authors have begun to (re)advocate the use of MDMA as a therapeutic tool (e.g. anxiety and posttraumatic stress disorders). Altogether, it is a confusing situation in which the media have become more and more interested with conflicting headlines like ‘Ecstasy, what it does to your brain’<sup>6</sup> and ‘Ecstasy Not Dangerous, Say Scientists’<sup>7</sup>. The retraction of a *Science* paper in which MDMA was reported to produce Parkinson’s like disease in monkeys treated with the drug added up to the confusion<sup>8,9</sup>. Amid this confusion, MDMA supporters are trying to bring MDMA to the clinic. In South Carolina MDMA is now being studied under experimental conditions as a psychotherapy adjuvant in the treatment of anxiety and posttraumatic stress<sup>10</sup>. MDMA supporters claim that studies showing cognitive deficiencies following ecstasy use are methodologically flawed, and that there is no proof that a few doses of the drug will cause harm<sup>10</sup>. It is therefore all the more important to summarize what we do know and what we do

not know about ecstasy's neurotoxicity. What follows is an overview of neuroimaging studies that have evaluated the potential neurotoxic effects of ecstasy on the human brain with a discussion focusing on technical aspects, conceptual issues, and future prospects. Although several neuroimaging studies have investigated whether use of ecstasy is associated with secondary, indirect, changes in post-synaptic serotonin receptor densities, brain microvasculature, cerebral glucose metabolic rate, and brain activation patterns, this review will concentrate on neuroimaging studies which have studied the effects on serotonin transporter densities (SERT), a known marker for serotonergic neurotoxicity, and levels of the neurometabolite *N*-acetylaspartate (NAA), a marker for non-specific neuronal loss, as will be discussed later. These two markers are selected because they are most directly linked to neural injury. In order to understand the rationale for neuroimaging studies in ecstasy users, a short description of the most important findings in animal research is unavoidable.

## ANIMAL DATA

MDMA-induced neurotoxicity has been demonstrated using a variety of experimental techniques at doses that approach or overlap equivalent doses used recreationally by humans. In these animal studies, serotonergic neurotoxicity is evidenced by losses in various markers unique to serotonergic neurons, such as serotonin, 5-hydroxyindolacetic acid (5-HIAA), tryptophan hydroxylase, and SERT<sup>11-19</sup>. Furthermore, it has been shown that MDMA-induced loss of serotonergic axonal markers is related to distal axonotomy (for review see<sup>20</sup>). These studies further show that the effects of MDMA are selective, damaging brain serotonergic axons. The effects of MDMA on serotonergic axons may be long-lasting since studies in non-human primates suggest that while some brain regions show evidence of complete recovery, other regions remain denervated up to seven years after treatment with MDMA<sup>21</sup>. Recently, it was noted that administration of MDMA to neonatal rats caused a persistent reduction of SERT in the neocortex (Meyer and Ali, 2002). This contrasts with studies in adult rats, in which soon after MDMA administration partial recovery is seen. These observations suggest that early administration of MDMA may cause permanent damage to the developing brain.

It has been shown that a single dose of 10 mg/kg produces marked transient depletions in serotonin and 5-HIAA in rat brain persisting for 1 week or longer<sup>22</sup>. Because primates are thought to be much more vulnerable to the neurotoxic effects of MDMA than rodents, a single dose of 5 mg/kg MDMA has been shown to produce long-lasting depletion of serotonin in monkey brain<sup>14</sup>. Using the principle of interspecies scaling, the equivalent known neurotoxic dose of MDMA in rats is 20 mg/kg, and 5 mg/kg in monkeys, which is approximately 96 mg for a 75 kg

individual<sup>23</sup>, suggesting that in most animal studies animals are treated with doses comparable to the recreational dose used by humans. A recent study observed that plasma concentrations of MDMA shown to produce lasting serotonergic deficits in squirrel monkeys overlap those used by recreational users and are two to three times higher than those found in humans administered a single 100–150 mg dose of MDMA in a controlled setting<sup>24</sup>. However, the regularly used dosing scheme, twice daily for 4 consecutive days, is not comparable. The recent observation in monkeys that after approximately 18 months of three times per week self-administration of on average 2 to 4 mg/kg MDMA there were no measurable decrements in serotonin, 5-HIAA, dopamine, or 3,4- dihydroxyphenylacetic acid (DOPAC) is therefore of particular interest<sup>25</sup>.

Finally, brain levels of dopamine and its metabolite are not reduced by lower doses of MDMA, but are depleted after higher doses<sup>15</sup>, suggesting that while MDMA is more toxic to serotonergic than to dopaminergic systems, it can also damage dopamine neurons.

In summary, MDMA administration at doses that approach those used recreationally by humans have consistently shown to cause selective injury to the serotonergic-system, and to the DA system at higher doses in animals. The effects are highly dependent upon age, dose, and interval between the administrations. Furthermore time and brain region play an important factor, because the effects are long-lasting in several brain regions, while others will show complete recovery over time. These are important aspects to take into account when conducting studies in human ecstasy users.

## HUMAN DATA

In humans the following markers for serotonergic neurotoxicity have been studied using *in vivo* neuroimaging techniques: (a) decrease in SERT detected by positron emission tomography (PET) and single photon emission computed tomography (SPECT), and (b) a decrease in the neurometabolite NAA, a marker for non-specific neuronal loss, detected by proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS). The studies, which have addressed these two markers in human ecstasy users will be discussed below.

The use of *in vivo* SERT densities as a marker for serotonergic neurotoxicity obtained using SPECT and PET has been validated in animals treated with MDMA. Previous PET and SPECT studies in monkeys and rats treated with MDMA have shown reductions in SERT densities, although SERT reductions were generally higher when studied using *ex vivo* binding studies or autoradiography, suggesting that PET and SPECT tend to underestimate the extent of serotonergic neurotoxicity<sup>26-29</sup>. The

use of NAA as a marker for detecting MDMA-induced neurotoxicity has not been validated in MDMA-treated animals.

## Decrease in SERT

### PET and SPECT studies in humans

SERT neuroimaging studies in human ecstasy users provide suggestive evidence that users of ecstasy are susceptible to ecstasy-induced neuronal damage. In these studies ecstasy's neurotoxic potential was investigated directly by studying the density of the SERT. The SERT is a structural element of the pre-synaptic serotonergic neuron, and has been shown to be a reliable marker of the integrity of the serotonergic neuron<sup>30,31</sup>. Different PET and SPECT radioligands have been developed for neuroimaging of SERT in the human brain. Because animal studies have already shown that MDMA-induced neurotoxicity is associated with loss of serotonergic axons, PET and SPECT are the most important imaging techniques for studying the potential neurotoxic effects of ecstasy on the SERTs in the living human brain.

The introduction of an increasing number of radioactive tracers and the development of special detecting systems, enable the detection of molecules *in vivo* and the production of functional images of brain chemistry. PET uses relatively short-lived positron-emitting isotopes (such as <sup>11</sup>C or <sup>18</sup>F), whereas SPECT utilizes radioligands with a longer half-life (such as <sup>123</sup>I and <sup>99m</sup>Tc). Spatial resolution of most recently developed PET systems is approximately 4 mm. The spatial and temporal resolution of SPECT is lower than that of PET. However, because of the lower costs of the less complex logistics and production of SPECT radiotracers, this technique is more widely available than PET. Due to the relatively long half-life of the SPECT tracers, SPECT offers the possibility to start the acquisition of data even many hours after injection at a moment that equilibrium of binding is reached.

There are important requirements for a good *in vivo* tracer for SERT, and therefore there are only a few radioligands which fulfil the minimal criteria and subsequently three tracers have been used to investigate the effects of ecstasy *in vivo*: for PET [<sup>11</sup>C]McN5652 and [<sup>11</sup>C]DASB; for SPECT [<sup>123</sup>I]β-CIT.

### PET studies

Trans-1,2,3,5,6,10-β-hexahydro-6-[4-(methylthio)phenyl] pyrrolo-[2,1- $\nabla$ ]isoquinoline ([<sup>11</sup>C]McN5652) is the first PET radioligand successfully developed in 1992 to label SERT in the living human brain<sup>32</sup>. *In vitro*, the active enantiomer (+)-[<sup>11</sup>C]McN5652 is a selective and potent inhibitor of serotonin uptake. The *in vivo* regional distribution of (+)-[<sup>11</sup>C]McN5652 in rats, baboons, and humans correlated with known regional concentrations of SERT, and the specific uptake of (+)-[<sup>11</sup>C]McN5652 is blocked after pre-treatment with the serotonin uptake blocker fluoxetine. In contrast, the brain

uptake of the inactive enantiomer (-)-[<sup>11</sup>C]McN5652 is relatively uniform across brain regions, representing non-specific binding. In 1998 a dual tracer PET study, in which both the (+) and the (-) isomer of [<sup>11</sup>C]McN5652 were administered, was carried out in human ecstasy users<sup>33</sup>. The purpose of the study was to compare [<sup>11</sup>C]McN5652-labelled SERT densities in human ecstasy users with SERT densities in control subjects. Nine males and four females who reported previous use of ecstasy were enrolled, along with nine male and six female control subjects. Participants agreed to abstain from use of psychoactive drugs for at least 3 weeks before the study. Distribution volumes (DVs), reflecting SERT densities were globally (in subcortical and cortical brain areas) decreased in ecstasy users, which correlated with the extent of previous ecstasy use. Taken in conjunction with the results of previous animal studies showing selective decreases in serotonergic axonal markers, such as SERT, after treatment with ecstasy<sup>28</sup>, this was the first report providing direct evidence that ecstasy users are susceptible to ecstasy-induced brain serotonergic neuronal injury. However, this study was limited by the use of a dual tracer approach to correct for non-specific binding in which the two tracers ((+)-[<sup>11</sup>C]McN5652 and (-)- [<sup>11</sup>C]McN5652) were not modelled simultaneously. The results showed such high variability in estimates of SERT densities (resulting in the use logarithmic transformations to permit statistical analyses), that it has been suggested that the results of the study reflected the difference in kinetics of the non-specific and free ligand and not SERT densities *per se*<sup>34</sup>.

Buchert and others on the other hand used the cerebellum as a reference region for non-specific binding in a large PET study<sup>35</sup>. SERT densities were measured in 117 subjects: 30 current ecstasy users, 29 former ecstasy users, 29 drug-naïve controls, and 29 polydrug control subjects. Participants agreed to abstain from use of psychoactive drugs for at least 3 days before the study. Current ecstasy users were scanned on average 3.5 weeks after their last ecstasy tablet (range 4–60 days), former users on average 1.4 years (range 29–1500 days). However, the eligibility criterion for the former users group was a minimum abstinence period of 20 weeks (140 days). It is therefore remarkable that subjects of the former users group were already scanned after a minimum of 29 days, and that there exists considerable overlap between the current and former users on this point. DVs were significantly reduced in the mesencephalon, thalamus, left caudate, hippocampus, occipital cortex, temporal lobes, and posterior cingulate gyrus of current ecstasy users compared with other groups. Reduction was more pronounced in female than in male subjects. There was no significant difference in SERT density among former ecstasy users, drug-naïve, and poly-drug control subjects, suggesting that the effects of ecstasy on SERT are reversible. However, subjects were scanned after a very short abstinence period of minimal 3 days. Because both MDMA and [<sup>11</sup>C]McN5652 bind to the SERT, one has to ensure that the SERT is available for [<sup>11</sup>C]McN5652 binding. Although the time point after MDMA administration at which the SERT is free to bind is unknown,

**Table 1:** Summary of SERT observations using PET and SPECT in heavy ecstasy users

	Sample size <i>N</i>	Exposure (pills)	Overall SERT	SERT occipital cortex
<b>PET</b>				
McCann et al. 1998*	29	880	↓ (SC+C)	Estimated -85% in males and females
McCann et al., 2005*	42	173	↓ (SC+C)	-54 & -68% in males and females
Buchert et al., 2004	117	831	↓ (SC+C) in females; in males only in occipital and temporal cortex	>> -10%
<b>SPECT</b>				
Semple et al., 1999	20	672	↓ (C only)	-8% in males
Reneman et al., 2001	69	530	↓ (SC+C) in females, not in males	NS in males, -16% in females

\*Estimated values from graphs

SC: Subcortical brain areas

C: cortical brain areas

?: unknown

previous studies in humans have applied abstinence periods of at least 3 weeks<sup>33,36</sup>. Because plasma levels of MDMA decline following a monoexponential model with a mean elimination half-life of about 8 h<sup>37</sup> in humans, the authors pointed out that within 3 days plasma levels drop far below 1% of the peak level. Furthermore, MDMA has a much lower affinity for the SERT than [<sup>11</sup>C] McN5652. Finally, exclusion of all subjects with an abstinence period of less than 2 weeks did not change the outcome of the study, except for the posterior cingulate gyrus and left caudate, suggesting that the SERT reductions reflect ecstasy-induced neurotoxicity and not acute pharmacological effects of MDMA<sup>35</sup>.

Recently, McCann and others replicated their [<sup>11</sup>C]McN5652 PET study in ecstasy users, using the cerebellum to control for non-specific binding<sup>38</sup>. A total of 23 ecstasy users and 19 control subjects were studied. The ecstasy users were scanned after a drug free interval of at least 2 weeks, but on average 4.7 months after their last ecstasy ingestion. Global DVs were significantly lower in ecstasy users. Twelve out of the 15 cortical and subcortical regions of interest showed reduced DVs in ecstasy users. Furthermore, a significant relationship was observed between global and regional [<sup>11</sup>C]McN5652 DVs and duration of abstinence, suggesting that SERT may recover over time. However, unlike their previous study and unlike the Buchert study, no reductions in SERT densities were observed in the midbrain and putamen<sup>35</sup>. This is puzzling because both brain regions are rich in SERT densities. It is well known that raphe nerve cell bodies are unaffected by MDMA<sup>21</sup>. However, an *ex vivo* SERT binding study in MDMA-treated rats has shown that although there was no significant reduction in SERT densities in the raphe nuclei, midbrain SERT (containing not only the raphe nuclei, but substantia nigra and superior colliculi as well) was significantly

SERT in midbrain	Moderate use	Gender	Reversibility	Correlation dose
Estimated -30%	NA	-	-	Life time dose
NS	NA	?	+	Typical dose
Estimated -30%	NA	+	+	Typical dose
NS in males	NA	Males only	+	Life time dose
NS in males, -13% in females	-	+	+	Life time dose

reduced when assessed using [ $^{123}\text{I}$ ] $\beta$ -CIT<sup>27</sup>. This suggests that the known sparing of the raphe nerve cell bodies by MDMA is not a good explanation for the lack of midbrain SERT reductions in the McCann study<sup>38</sup>. However, it is well known that the serotonergic neurotoxic effects of MDMA are dose-dependent. This is probably also true in humans because all studies have observed an association between SERT density and extent of ecstasy exposure; either lifetime dose or typical dose (Table 1). Because ecstasy users in the McCann 2005 study had an average exposure five times lower than those in the 1998 study (173 vs 880 pills, respectively), this may also be an explanation for the discrepancy in midbrain SERT reductions between the two studies. In addition, there is a much higher variability in DVs of the 2005 McCann study for midbrain and putamen than in their previous study from 1998, which may explain the absence of a significant effect of ecstasy on midbrain SERT. The authors suggest that the high variability probably reflects different durations of abstinence (resulting in non-uniform recovery).

Along with [ $^{11}\text{C}$ ]McN5652, McCann *et al.* studied a new promising PET ligand for labeling SERT, [ $^{11}\text{C}$ ]DASB ([ $^{11}\text{C}$ ]amino-4-(2-dimethylaminomethylphenylsulfanyl)b enzonitrile), which was tested in ecstasy-using subjects and controls<sup>38</sup>. There was a high correlation between the two tracers, and no significant differences between the tracers were noted. Using [ $^{11}\text{C}$ ]DASB, similar results to [ $^{11}\text{C}$ ]McN5652 were obtained. The authors had hoped that because [ $^{11}\text{C}$ ]DASB has a greater specific-to-non-specific equilibrium activity ratio than [ $^{11}\text{C}$ ]McN5652, as well as a measurable plasma free fraction for use in tracer modelling, differences in regions with relatively low SERT densities such as the neocortex could be detected. However, a previous study has shown that the advantage of [ $^{11}\text{C}$ ]DASB over [ $^{11}\text{C}$ ]McN5652 is mainly related to a shorter

scanning time <sup>39</sup>. In addition, pretreatment with paroxetine displaced both ligands primarily from regions with high SERT densities <sup>29</sup>. Without correcting for multiple comparisons, no significant reductions in binding of [<sup>11</sup>C]McN5652 or [<sup>11</sup>C]DASB were observed in cortical regions <sup>29</sup>, suggesting that neither [<sup>11</sup>C]McN5652 nor [<sup>11</sup>C]DASB may be suitable in studying the effects of ecstasy in brain regions relatively devoid of SERT such as the neocortex, as previously noted also by others <sup>40</sup>. This is a problem also encountered with [<sup>123</sup>I]β-CIT SPECT, as will be discussed below.

### ***SPECT studies***

The cocaine analogue 2β-carbomethoxy-3β-(4-iodophenyl)tropane β-CIT is presently the best studied SPECT tracer for labeling of SERT. [<sup>125</sup>I]β-CIT binds with high affinity to both dopamine transporters (DAT) and SERT <sup>41</sup>. The *in vivo* regional distribution of [<sup>123</sup>I]β-CIT in rats, monkeys, and humans well correlates with known regional concentrations of SERT. The specific uptake of [<sup>123</sup>I]β-CIT in the striatum is primarily associated with DA transporters, since it is blocked by the selective DA reuptake inhibitor GBR 12,909 but not by selective serotonin reuptake inhibitors <sup>42</sup>. In contrast, uptake of β-CIT in serotonin-rich brain regions, such as the brainstem, thalamus, and cerebral cortex, can be blocked by serotonin reuptake inhibitors <sup>42-44</sup>. Thus, these studies indicate that in selected brain areas, e.g. brainstem, thalamus, cerebral cortex, and other regions in which SERT densities far exceed those of DAT, it is possible to estimate SERT densities using [<sup>123</sup>I]β-CIT.

*Ex vivo* and *in vitro* studies in animals have shown that [<sup>123</sup>I]β-CIT adequately detects changes in cortical as well as subcortical SERT densities secondary to serotonergic neurotoxicity, although cortical measurements must be interpreted with caution <sup>26,27,45,46</sup>.

Several studies have been conducted using [<sup>123</sup>I]β-CIT SPECT to study the effects of ecstasy on human brain serotonergic system. Semple and colleagues observed decreased [<sup>123</sup>I]β-CIT binding only in the cerebral cortex (particularly prominent in the primary sensory cortex) of ten male heavy ecstasy users as compared to ten well-matched controls <sup>47</sup>. Reductions in binding inversely correlated with time since last ecstasy use, and correlated positively with estimated lifetime dose. There are, however, several problems associated with this study <sup>48</sup>. Subjects were asked to abstain from psychoactive drugs for 1 week, and were scanned on average after an abstinence period of 2.6 weeks. One cannot totally exclude that the results are at least in part influenced by acute pharmacological effects of ecstasy, as discussed above for the Buchert study <sup>35</sup>. Furthermore, [<sup>123</sup>I]β-CIT SPECT scans were acquired 90 minutes post-injection of the radiotracer. However, [<sup>123</sup>I]β-CIT does not reach near-equilibrium conditions earlier than about 4 h post-injection <sup>49</sup>. At scanning times this early, factors related to radioligand delivery and washout, rather than SERT binding *per se*, play a prevalent role in determining specific [<sup>123</sup>I]β-CIT binding to SERTs.

Using [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT, we studied the effects of ecstasy in three different subgroups of 54 ecstasy users and 15 controls<sup>36</sup>. Fifteen polydrug, but ecstasy-naive controls, 15 moderate, 23 heavy, and 16 former ecstasy users were enrolled in the study. Eligibility criterion for the former ecstasy group was a minimum of 50 tablets lifetime but they should have stopped using ecstasy for at least 1 year. Current ecstasy users (moderate and heavy) were scanned on average 3.0 months after their last ecstasy tablet, former users on average 2.4 years. Subjects were scanned after a minimum drug-free interval of at least 3 weeks. Subjects were recruited from the same community sources, and thus well matched for age, gender distribution, and psychosocial factors. Significant decreases in overall binding ratios in female, but not in male heavy ecstasy users, were observed suggesting that females may be more susceptible than males to the neurotoxic effects of ecstasy. It was also observed that in most, but not all, brain regions of female ex-ecstasy users SERT densities were comparable to controls. Finally, moderate ecstasy use was not associated with significant reductions in SERT densities, although reductions were observed in the parieto-occipital cortex and occipital cortex of moderate users, brain regions, which seem to be particularly sensitive to ecstasy's effects. Evidence is accumulating that the consequences and mechanisms of ecstasy (ab)use are not identical in males and females. In line with our observations, McCann and co-workers observed greater reductions in 5-HIAA concentrations in the cerebrospinal fluid of female compared to male ecstasy users<sup>3</sup>. Furthermore, Liechti and co-workers reported more pronounced subjective responses to ecstasy in females than in males<sup>50</sup>. These observations support the findings by us and by Buchert that females may be more susceptible than males to the (neurotoxic) effects of ecstasy<sup>35,36</sup>. Also with respect to other drugs of abuse it has been noted that the consequences and mechanisms are not identical in males and females. The aetiology of these gender differences is unknown, but may be related to differences in innate hormonal profiles<sup>51</sup>, volume and morphology of certain brain structures<sup>52</sup>, monoaminergic neurotransmission or to the effect of other drugs of abuse such as cannabis or alcohol or the effect of a functional polymorphism in the gene encoding SERT polymorphism<sup>53</sup> as discussed later.

Whether SPECT imaging with [ $^{123}\text{I}$ ] $\beta$ -CIT is sensitive enough to measure the density of serotonin transporters in areas of the cerebral cortex is controversial, and subject to debate<sup>48,54</sup>. It has been argued that the region of choice when studying SERT densities is the raphe area of the brainstem because the thalamus may have a substantial admixture of noradrenaline transporters<sup>43</sup> and because it is difficult to avoid scattered radiation from the much greater accumulation of activity in the striatum when studying the thalamus. Therefore, we recently investigated the value of [ $^{123}\text{I}$ ] $\beta$ -CIT in assessing SERT densities<sup>55</sup>. In a double-blind, placebo-controlled, cross-over design the effect of the selective SSRI citalopram on [ $^{123}\text{I}$ ] $\beta$ -CIT binding was assessed in cortical as well as subcortical brain regions. After citalopram

treatment [ $^{123}\text{I}$ ] $\beta$ -CIT binding was reduced in midbrain and (hypo)thalamus region, along with cortical brain regions, although statistical significance was only reached in several cortical areas using voxel-by-voxel analysis. The results of this study suggest that [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT is a valid technique in studying SERT densities in serotonin-rich brain regions such as the midbrain and (hypo)thalamus. However, even though [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT may be used to measure cortical SERT densities, these cortical measurements must be interpreted with caution, as has been shown already for [ $^{11}\text{C}$ ]DASB and [ $^{11}\text{C}$ ]McN5652 PET.

Another problem associated with [ $^{123}\text{I}$ ] $\beta$ -CIT is its affinity for both serotonin and DAT. The midbrain, thalamus, and cortex also contain DAT besides SERT. However, displacement studies in animals <sup>43,46</sup> have shown that binding of  $\beta$ -CIT is predominantly associated to SERT in these brain regions. Furthermore, since we <sup>56</sup> and Semple <sup>47</sup> did not observe reductions in striatal [ $^{123}\text{I}$ ] $\beta$ -CIT binding ratios (obtained 24 h p.i. of the radiotracer) between heavy ecstasy users and controls it can be concluded that observations in ecstasy users most likely reflect differences in SERT and not DAT.

## Decrease in NAA

### *<sup>1</sup>H-MRS studies*

MR spectroscopy is an important supplement to MRI for medical diagnosis in a variety of diseases. MRS is based on the same physical principles but offers unique biochemical information from various organs and tissues and is therefore increasingly applied to improve tissue characterization in normal and pathological states. The reduction of the amino acid NAA detected by <sup>1</sup>H-MRS represents a robust but non-specific marker for neuronal loss or dysfunction <sup>57</sup>. Although the use of NAA has not been previously validated for detecting ecstasy-induced neurotoxicity, unlike SERT, PET, and SPECT, NAA has been shown to be predominantly localized to neurons, axons, and dendrites within the central nervous system <sup>58</sup>. Studies of diseases known to involve neuronal and/or axonal loss (infarcts, brain tumors, seizure foci, multiple sclerosis plaques, for example) have uniformly shown NAA to be decreased. Animal models of chronic neuronal injury have also been shown to give good correlations between NAA levels (as measured by MRS) and *in vitro* measures of neuronal survival <sup>59,60</sup>. In addition to NAA, myo-inositol (mI, a possible glial marker), choline (Cho), and creatine/phosphocreatine (Cr) can be assessed. Determining NAA changes in relation to Cr is commonly employed, because Cr remains stable in a variety of brain diseases and can thus function as some kind of calibration.

Chang and colleagues reported the first findings on <sup>1</sup>H-MRS spectra obtained in 22 ecstasy users and 37 controls, who had to abstain from psychoactive drugs for at least 2 weeks <sup>60,61</sup>. Normal NAA levels were observed in ecstasy users, but mI and mI/Cr levels were increased in the parietal white matter of ecstasy users. The

cumulative lifetime ecstasy dose showed significant effects of mI in the parietal white matter and the occipital cortex. The normal NAA levels suggest neuronal integrity in ecstasy users, whereas increased mI may reflect increased glial content, possibly reflecting ongoing repair processes.

In contrast, we reported decreased NAA/Cr and NAA/Cho levels in the frontal cortex of 15 male ecstasy users, studied at least 1 week after the last ecstasy tablet taken, as compared to 12 gender and age matched control subjects<sup>62</sup>. Furthermore, a significant association was observed between the extent of previous ecstasy use and NAA/Cr or NAA/Cho ratios in the frontal cortex. Discrepancies between the study by Chang and that of Reneman, may be attributed in part to age-associated differences between both studies. In the Reneman study, subjects (both ecstasy users and controls) were on average younger with a smaller age range. However, precise quantification of 'near-water' resonance peaks is difficult in water-suppressed <sup>1</sup>H-MRS, and may therefore also account for the discrepancy between the studies.

In an exploratory study, hippocampal <sup>1</sup>H-MRS spectra of five ecstasy users were compared with those of controls with no history of substance abuse<sup>63</sup>. No differences between users and controls were observed. Furthermore, Daumann and colleagues recently compared <sup>1</sup>H-MRS spectra of 13 ecstasy users with 13 controls<sup>64</sup>. No differences were observed in cortical NAA/Cr ratios between the two groups, whereas only a tendency towards lower NAA/Cr ratios was observed in the left hippocampus of ecstasy users. The discrepancy between these studies and that of Reneman is most likely attributed to the much higher ecstasy exposure of the subjects in the Reneman study (mean dose of more than 700 tablets).

## DISCUSSION

The above-mentioned studies have all found reductions in SERT density in heavy ecstasy users with the use of different techniques and different radioligands. In all but one study<sup>47</sup> subcortical as well as cortical reductions were observed. The reductions seem to be dependent on dose (lifetime dose and typical dose used), as well as on gender. In two out of the three studies in which gender was taken into account, a significant effect of gender was observed in which females were found to be more vulnerable than males<sup>35,36</sup>. In the third study that did not find gender differences<sup>33</sup>, gender differences may have been observed if more women had been included (only six women were enrolled). Furthermore, in four out of five studies, an association was observed between SERT densities and duration of abstinence, consistent with studies in animals which demonstrate recovery of serotonergic axonal terminals in some but not all brain regions<sup>21</sup>. With respect to the use of <sup>1</sup>H-MRS in the context of studying the neurotoxicity of ecstasy, it may very well be that

only very high levels of ecstasy use may cause detectable decrements in NAA levels in the brain. Therefore, <sup>1</sup>H-MRS appears to be a less sensitive technique for the study of the potential neurotoxicity of ecstasy.

There are also some puzzling discrepancies between the different PET and SPECT studies. For instance, occipital differences in SERT densities in ecstasy users studied with [<sup>123</sup>I]β-CIT SPECT by Semple <sup>47</sup> and Reneman <sup>36</sup> (in the order of 10%) are similar. However, this is not the case when compared with McCann <sup>33,38</sup> (estimated from graph at -54% to -85%; Table 1) using [<sup>11</sup>C]McN5652 and [<sup>11</sup>C]DASB PET. Previous PET and SPECT studies in animals have shown that PET and SPECT underestimate the true extent of SERT loss by approximately 40–50% <sup>26,29</sup>. In MDMA-treated baboons, reductions in the order of 30% in most regions were observed with a maximum of 42% in the occipital cortex <sup>29</sup>. Clearly, the use of the cerebellum as a reference region for non-specific binding may explain the discrepancy because the cerebellum is not completely devoid of serotonin <sup>65</sup>. This will result in an overestimation of SERT densities, and consequently an underestimation of ecstasy's neurotoxic effects <sup>65</sup>. In addition, the use of more selective radioligands for the SERT will further help overcome the discrepancy to some extent between *in vivo* and *in vitro* observations. However, the reasons for the discrepancy between the Buchert <sup>35</sup> and McCann study <sup>38</sup> with respect to SERT reductions observed in the occipital cortex (-10 vs. -54%) remain unclear. Also the difference between the two McCann studies with respect to the midbrain SERT (no significant reduction vs. 30% reduction) is difficult to explain. They probably relate to the fact that different models were used to estimate SERT binding, and problems associated with analysis of SERT in the midbrain region (subject movement during scanning, spill over from the thalamus) as well as extent of previous exposure to ecstasy, as previously discussed.

It should be kept in mind, that it is an assumption that a decrease in SERT density directly reflects axonal loss. One factor that may influence results of imaging studies of the SERT is whether or not the binding of the radiotracer is sensitive to changes in endogenous intrasynaptic serotonin levels. A ligand sensitive to competition by endogenous serotonin may enable the measurement of acute fluctuations of serotonin, such as stress. A ligand insensitive to competition provides a more reliable measure of SERT unaffected by levels of serotonin. This seems to be particularly true for [<sup>123</sup>I]β-CIT SPECT, and not as much for [<sup>11</sup>C]DASB (and possibly [<sup>11</sup>C]McN5652) PET), as β-CIT can be displaced by endogenous serotonin <sup>66</sup>, whereas [<sup>11</sup>C]DASB binding to SERT declined after acute reduction of 5-HT levels <sup>67</sup>. In addition, recent *in vitro* studies have observed that SERTs are trafficked between the cell membrane and the intracellular compartment. Both sequestration and reduced protein synthesis are thought to be involved in a homeostatic loop in which SERT density on the cell membrane is linked to synaptic serotonin concentrations <sup>68</sup>. Acutely, serotonin will prevent sequestration of SERT <sup>68</sup>. However, if serotonin concentrations are

low for a long period of time, homeostatic mechanisms may reduce synthesis of the SERT protein as a 'neuroadaptive' response to low serotonin levels, in which low levels of serotonin will lead to increased sequestration and reduced synthesis of the SERT<sup>69</sup>. Although a variety of animal studies demonstrated that reductions in immunostained serotonergic axons and axon terminals are associated with decreases in serotonergic axonal markers (such as SERT), there is an ongoing debate whether SERT reductions after MDMA administration represent a neuroadaptive response, a functional downregulation of the SERT, or reflect toxic loss of serotonergic terminal fibres. In this respect it is of interest to note that administration of the irreversible tryptophan hydroxylase inhibitor p-chlorophenylalanine produced significant and profound reductions in cortical serotonin and 5-HIAA levels but not SERT, whereas the serotonergic neurotoxin p-chloroamphetamine reduced not only serotonin and 5-HIAA but SERT as well<sup>70</sup>. This study indicates that SERT reductions after p-chloroamphetamine administration are not a consequence of prolonged serotonin depletions, but reflect p-chloroamphetamine induced brain serotonergic neurotoxicity, and led the authors to conclude that SERT is a reliable marker for 5-HT neurotoxicity. However, the effects of MDMA on SERT internalization have not been previously studied (paper by Boot and others<sup>71</sup> was retracted by the authors).

In view of the problems associated with measuring SERT in SERT-low regions (such as the neocortex) there is a striking similarity between the different PET and SPECT studies: in all five studies reductions were observed in cortical SERT. Animal studies have shown that the neocortex is particularly sensitive to the neurotoxic effects of MDMA. PET studies in animals treated with MDMA have shown SERT reductions of about 30% in most brain regions, with a maximum of 42% in the occipital cortex<sup>29</sup>. Although SERT measurements with PET and SPECT in the neocortex should be interpreted with caution, the large preclinical literature demonstrating toxicity of MDMA toward serotonergic axons, which is especially profound in cortical brain regions, together with the similarity of the neuroimaging studies in humans, provides at least suggestive evidence that humans who use ecstasy are not only subject to subcortical, but also to cortical loss of SERT.

It is important to note that the presently discussed studies are limited by a number of factors. Their conclusions heavily depend upon previous results in experimental animal studies showing MDMA-induced serotonergic lesions. Furthermore, studies in humans are clearly subject to ethical and methodological constraints as discussed in detail elsewhere<sup>72</sup>. Consequently, until now most studies conducted in humans have had a retrospective design, in which evidence is indirect and differs in the degree to which any causal links can be implied between ecstasy use and neurotoxicity. Clearly, to definitively establish a causal link between observed neurotoxic changes and ecstasy use, an experimental study design is needed. However, given that the drug is illicit, has potential neurotoxicity, and has resulted in some fatalities, it is very difficult to

get medical ethical approval for such a study. One possible approach would be to assess people both before and after they took ecstasy. Neuroimaging techniques may be very helpful in providing such longitudinal studies in human ecstasy users <sup>73</sup>.

Although PET and SPECT studies may be limited by several factors (e.g. the low cortical uptake and the use of a non-optimal reference region (cerebellum)) experimental studies in small laboratory animals and non-human primates have shown that these techniques can adequately detect ecstasy-induced reductions in SERT densities in several serotonin-rich brain regions, and to some extent also cortical SERT. 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 in the same direction. It is therefore noteworthy that PET/SPECT evaluations of 5-HT<sub>2</sub> receptor densities, FDG PET, functional MRI (fMRI), perfusion and diffusion MRI, and cognitive studies are all indicative of alterations of brain (serotonin) structure and function in ecstasy users. Without doubt more optimal techniques to evaluate ecstasy-induced neuronal loss will emerge in the future. For instance, more selective radioligands for the SERT are being developed for SPECT that may be more sensitive in detecting ecstasy-induced neuronal loss, such as for instance (2-((2-((dimethylamino)methyl)-phenyl)thio)-5-iodophenylamine) ([<sup>123</sup>I]ADAM), which has high binding affinity and selectivity toward SERT <sup>74,75</sup>. Until then, future PET and SPECT studies will be needed to investigate the sensitivity and specificity of SPECT and PET in detecting ecstasy-induced neuronal loss. Furthermore, other techniques such as fMRI, perfusion and diffusion MRI may come to play an important role in the future. The combined use of these techniques may provide additional insights into the neurotoxicity of ecstasy 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 analysis was performed, automatic voxel-based analysis may be more powerful than, but consistent with, ROI analysis, and seems to be a valuable tool in detecting small differences between ecstasy users and controls.

Future studies will have to find out whether neurotoxic effects in heavy ecstasy users tested to date also occur in less frequent users. Some have argued that even a single dose of ecstasy may be neurotoxic in human beings <sup>23,76</sup>. Ecstasy users may be studied prospectively to shed light on the fate of damaged serotonergic neurons with age, and whether dysfunction (e.g. memory loss) resolves with abstinence or increases with age. More studies should be conducted combining neuroimaging studies with neuropsychological assessments to study links between brain damage and for example memory loss. More studies should be conducted focusing on other systems than the serotonergic system to increase our understanding on the effects of ecstasy and subsequent compensatory mechanisms in the brain. Because SERT

plays a key element in the regulation of synaptic serotonin transmission it may be important to control for the potential covariance effect of a functional polymorphism in the gene encoding SERT polymorphism when studying the effects of ecstasy. In line with this, it was recently observed that ecstasy users carrying the short allele are at particular risk for emotional dysfunction<sup>77</sup>, although we did not observe such an effect<sup>78</sup>. Because sample sizes were small in the latter two studies, more studies are needed to investigate a potential genetic basis for differences in vulnerability to ecstasy's neurotoxic effects. Finally, ecstasy is frequently taken in combination with other drugs, such as amphetamine, cocaine, cannabis, and ethanol. The effect of these combinations on ecstasy's serotonergic neurotoxicity are not known, although some studies in animals have been performed. Recently a partial protective effect of co-administered cannabinoid receptor agonists on MDMA-induced serotonin depletion and long-term anxiety<sup>79</sup> has been shown, whereas the combination of MDMA with ethanol may result in long-term consequences on pre-synaptic modulation of hippocampal serotonin release<sup>80</sup>. It is therefore important that we gain more insight into the combined effects of these drugs on ecstasy's neurotoxic potential.

If indeed ecstasy leads to serotonergic neuronal injury the health implications may be considerable, in that ecstasy may be responsible for early or late neuropsychiatric morbidity. Neuroimaging techniques will greatly contribute to our understanding of ecstasy's short- and long-term effects in the human brain. The fact that all these techniques are non-invasive and most of them can be used repeatedly in the same subject is a very critical feature.

#### *What do we know?*

- Heavy users of ecstasy have lower subcortical SERT densities than non-users.
- This effect is dose-dependent and probably transient.

#### *What remains to be determined?*

- Most studies have had a retrospective design, in which evidence is indirect and differs in the degree to which any causal links can be implied. Longitudinal studies in human ecstasy users are needed to draw definite conclusions.
- Whether females are more susceptible than males needs to be reconfirmed.
- Whether individuals with a relatively low ecstasy exposure also demonstrate loss of SERT needs to be determined, along with the clinical implications thereof.
- Whether evaluation of NAA levels using <sup>1</sup>H-MRS is useful in evaluating heavy ecstasy users.
- Confounding effects of age, SERT polymorphism, other drugs of abuse, and dosing scheme on ecstasy's neurotoxic potential.
- Will more selective SERT radioligands confirm previously made observations in ecstasy users?

### *Minimum standards for good experimental design*

- Use of polydrug controls.
- Matching for age and gender.
- Minimum duration of abstinence prior to neuroimaging study (at least 10 days).
- Detailed description of drug history, and possibly hair analysis.
- Clear description of eligibility criteria for specific groups.

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CHAPTER

4

# Validity of *In Vivo* [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT in Detecting MDMA-Induced Neurotoxicity in Rats

European Neuropsychopharmacology 2004; 14: 185-189

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## ABSTRACT

### Background

With [ $^{123}\text{I}$ ] $\beta$ -CIT, a radioligand that binds with high affinity to SERTs and dopamine transporters (DATs), it has become possible to use single-photon emission computed tomography (SPECT) to assess serotonin transporter (SERT) densities in the living brain.

### Aim

This study investigated the validity of a high-resolution pinhole SPECT system, with [ $^{123}\text{I}$ ] $\beta$ -CIT as a radiotracer, to detect 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy")-induced loss of SERTs in the living rat brain.

### Material and Methods

*In vivo* striatal and thalamic [ $^{123}\text{I}$ ] $\beta$ -CIT binding ratios, representing specific binding to dopamine and serotonin transporters, respectively, were determined 7 days before as well as 10 days after treatment of rats with neurotoxic doses of MDMA using SPECT. At the end of the experiment, radioactivity ratios were also determined *ex vivo*, and compared to control data.

### Results

Both *in vivo* and *ex vivo*, thalamic, but not striatal, uptake ratios were statistical significantly reduced after MDMA treatment.

### Conclusions

These data show that [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT may be able to detect MDMA-induced loss of SERTs. Therefore, this may be a promising technique to perform serial studies on MDMA-induced serotonergic neurotoxicity in living small animals.

## INTRODUCTION

3,4-Methylenedioxymethamphetamine (MDMA, “Ecstasy”) is a recreational drug of abuse frequently used among young adults. In line with a human necropsy study <sup>1</sup>, neurotoxic effects of MDMA on the serotonergic system have been described extensively in MDMA-treated animals, as evidenced by reductions in various markers unique to serotonin (5-HT) axons, including density of 5-HT transporters (SERTs) <sup>2</sup>. Since the SERT is located on the terminals of presynaptic axons of 5-HT neurons, it is considered to be a reliable marker of 5-HT neurons.

With [ $^{123}\text{I}$ ] $\beta$ -CIT, a radioligand that binds with high affinity to SERTs and dopamine transporters (DATs), it has become possible to use single-photon emission computed tomography (SPECT) to assess SERT densities in the living brain. The use of [ $^{123}\text{I}$ ] $\beta$ -CIT in detecting 5-HT lesions has been validated in *in vitro* and *ex vivo* studies in rodents <sup>3-5</sup>. In addition, one SPECT study has been conducted to validate the *in vivo* use of [ $^{123}\text{I}$ ] $\beta$ -CIT to detect MDMA-induced loss of SERTs in the SERT-rich midbrain/hypothalamic region in a monkey brain <sup>5</sup>. In humans, a [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT study by Reneman and co-workers <sup>6</sup> has shown decreased [ $^{123}\text{I}$ ] $\beta$ -CIT binding in SERT-rich brain regions such as midbrain and thalamus in females with a history of heavy ecstasy use. In male ecstasy users, however, no alteration of [ $^{123}\text{I}$ ] $\beta$ -CIT binding in SERT-rich brain regions were found <sup>6,7</sup>. Reductions of SERT in cortical brain regions, with relatively low concentrations of SERTs, were found in male <sup>7</sup> and female <sup>6</sup> ecstasy users.

An animal model of the serotonergic system is of intense interest to study experimentally the influence of different conditions (e.g., temperature and dosing patterns) on neurotoxic effects of MDMA. Moreover, the presumed neuroprotective properties of different drugs, such as serotonin transporter reuptake inhibitors, could be tested experimentally. Due to recent developments it is now possible to image rat brains with high-resolution using positron emission tomography (PET) (for review see <sup>8</sup>) and SPECT <sup>9,10</sup>. One of the most compelling advantages of using *in vivo* imaging in rats over many *ex vivo* techniques is the fact that the rat can survive the study and consequently serial studies within the same rat can be performed.

The recent development of a high-resolution single pinhole SPECT camera in our laboratory <sup>11</sup> has made it possible to image monoamine transporters *in vivo* in small animals. Studies by Booij *et al.* <sup>12</sup> have shown that this technique is able to detect reductions in striatal DAT densities in rats.

The objective of the present study was to investigate whether the small animal SPECT scanner is able to detect MDMA-induced loss of SERTs in the living rat brain using [ $^{123}\text{I}$ ] $\beta$ -CIT as a radiotracer.

## Experimental procedures

Seven days before treatment with MDMA, a baseline SPECT scan was made. Ten days after final MDMA treatment a post-treatment scan and *ex vivo* biodistribution studies were performed. In addition, *ex vivo* biodistribution studies were performed in a control group.

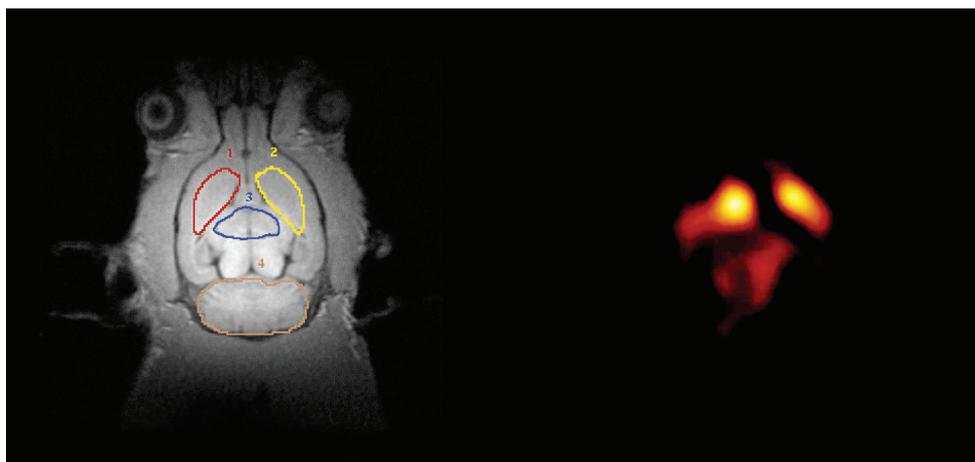
### Animals and drug treatment

Male Wistar rats ( $N = 11$ , obtained from Harlan, Horst, The Netherlands) weighing 200-250 g were used for this study. The rats were housed in a temperature-controlled environment and were on a 12-h light/12-h dark cycle with free access to food and water. The local Animal Care Committee approved the experiments. MDMA hydrochloride (certified reference compound, purity >98.9%) was obtained from The Netherlands' Forensic Institute. A frequently used and known neurotoxic dose regimen in Wistar rats is 20 mg/kg subcutaneously (s.c.) twice a day during four days<sup>2</sup>. However, this schedule was abandoned because 2 out of 6 rats died after the first treatment (macroscopic examination showed that one rat died of multiple cerebral haemorrhages, while the cause of death of the other rat could not be detected). The resulting 4 rats were treated with MDMA 20 mg/kg once a day on the first two days, 10 mg once a day on the third day, and 10 mg/kg twice a day on the fourth day.

### *In vivo* SERT imaging, single-pinhole SPECT camera, MRI

<sup>123</sup>I labeling of  $\beta$ -CIT was performed by oxidative radioiododestannylation (Radionuclide Center, Vrije Universiteit, Amsterdam, The Netherlands) of the trimethylstannyl precursor (specific activity >185 MBq/nmol; radiochemical purity >97%). Rats were injected with approximately 75 MBq [<sup>123</sup>I] $\beta$ -CIT in the tail vein. Scanning was started 2 h p.i., when equilibrium of the SERT binding in rats is approached<sup>13</sup>. During scanning, rats were anaesthetised by a mixture of ketamine-HCL and xylazine i.m. The SPECT system has previously been described extensively<sup>11</sup>. In the present study, a pinhole with a 2-mm aperture was used, resulting in a spatial resolution of 2.6 mm full-width at half-maximum (FWHM). Data acquisition included 100 projections of 30 s each. All experiments were acquired, in a step-and-shoot fashion, with a 20% energy window around 159 keV in a 128x128 matrix, and a radius of rotation of 45 mm. SPECT studies were reconstructed and analysed using HERMES application software (Nuclear Diagnostics, Stockholm, Sweden). Image analysis was performed using region of interests (ROIs). ROIs for striatum, thalamus and cerebellum were drawn on high-resolution MR images of a rat brain, performed with a 1.5 Tesla scanner (GE Signa-Lx), using a T1 weighted 3 dimensional fast-spoiled gradient echo sequence. For this purpose, a special receive-only surface coil was constructed with a diameter of 20 mm, fixed on a Delrin® support (Flick consulting,

The Netherlands). A FOV of 4x4 cm and a slice thickness of 0.7 mm in a 256x160x46 matrix were used. The ROIs were positioned without changing the size and form, on three and two consecutive SPECT slices, visualising most intense striatal and thalamic uptake, respectively, with help of the anatomical information from both MR images as well as a rat brain atlas. MRI anatomical information was used to position ROIs over two consecutive SPECT slices containing cerebellar uptake. [ $^{123}\text{I}$ ] $\beta$ -CIT binding in the striatum and thalamus was analysed using the ratio of total binding in the ROI divided by non-specific binding (=binding in cerebellum).



**Figure 1:** MR and [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT image of horizontal slices of rat brain at the level of the striatum with ROI template of right striatum (1), left striatum (2), thalamus (3), and cerebellum (4). The level of radioactivity is colour encoded from low (black) through medium (yellow) to high and scaled to the maximum of the study. (See page 309 for colour illustration)

### *Ex vivo* SERT binding studies

Control rats ( $N = 5$ ) were injected i.v. with approximately 3.7 MBq [ $^{123}\text{I}$ ] $\beta$ -CIT. Three hours after injection of [ $^{123}\text{I}$ ] $\beta$ -CIT in control rats and directly after [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT scanning (also 3 h after injection) in MDMA-treated rats (10 days after MDMA treatment,  $N = 4$ ), animals were sacrificed by bleeding via heart puncture under anaesthesia. The brains were quickly removed and striatum, thalamus and cerebellum were dissected and weighed. Radioactivity in each region was assayed in a gamma counter as described earlier<sup>14</sup> and expressed as a percentage of the injected dose, multiplied by the body weight per gram tissue weight (% ID  $\times$  kg/g tissue). Additionally, binding ratios in the ROI versus non-specific binding (in cerebellum) were calculated.

## Statistics

Results of the *in vivo* SPECT study of the remaining four rats were analysed using a Student *t*-test for paired samples. Data from *ex vivo* SERT biodistribution studies were analysed using a Student *t*-test for two independent samples. Statistical significance was defined as  $p < 0.05$ .

## RESULTS

MR images showed clear visualisation of striatum, thalamus, cortex and cerebellum of the rat brain (Figure 1). On the SPECT images, [<sup>123</sup>I]β-CIT binding was clearly visible in the thalamus, a brain region known to have high concentrations of SERTs (Figure 1). In addition, high accumulation of [<sup>123</sup>I]β-CIT was visible in the striatum, predominantly reflecting binding to DATs, whereas cerebellar uptake was very low. Uptake ratios before MDMA treatment were 1.64 and 2.11 in thalamus and striatum, respectively. After MDMA treatment, the thalamic binding ratio of [<sup>123</sup>I]β-CIT was

**Table 1:** *ex vivo* and *in vivo* binding of [<sup>123</sup>I]β-CIT in striatum, thalamus and cerebellum of the rat brain

	Striatum		Thalamus		Cerebellum	
	Control (N=5)	MDMA (N=4)	Control	MDMA	Control	MDMA
<i>Ex vivo</i>	74.21	54.73	16.78	10.31	4.56	3.49
(% ID	51.79	69.63	17.25	6.73	4.71	3.59
× kg/g	88.80	63.89	21.92	6.70	7.24	3.18
tissue)	89.67	87.67	19.50	10.19	5.07	4.10
	83.17		20.80		4.74	
Mean	77.53 ± 15.65	68.98 ± 13.89	19.25 ± 2.22	8.48 ± 2.04 <sup>†</sup>	5.26 ± 1.12	3.59 ± 0.38 <sup>†</sup>
<i>Ex vivo</i>	16.27	15.68	3.68	2.95		
ratios*	11.00	19.40	3.66	1.87		
	12.27	20.09	3.03	2.11		
	17.69	21.38	3.85	2.49		
	17.55		4.39			
Mean	14.95 ± 3.12	19.14 ± 2.45	3.72 ± 0.49	2.35 ± 0.47 <sup>†</sup>		
	<b>before MDMA</b>	<b>after MDMA</b>	<b>before MDMA</b>	<b>after MDMA</b>		
<i>In vivo</i>	2.12	1.78	1.81	1.16		
ratios*	1.74	1.89	1.45	1.14		
	2.31	2.48	1.71	1.56		
	2.26	1.86	1.59	1.29		
Mean	2.11 ± 0.26	2.00 ± 0.32	1.64 ± 0.16	1.29 ± 0.19 <sup>‡</sup>		

\* ratios are expressed as uptake in the region of interest divided by cerebellar uptake

<sup>†</sup> significant difference from control (Student *t*-test)

<sup>‡</sup> significant difference from before MDMA treatment (paired Student *t*-test)

significantly reduced by 21% ( $p = 0.044$ ), whereas the striatal binding ratio did not change significantly (-2%,  $p = 0.534$ ) (Table 1).

*Ex vivo* SERT biodistribution studies in control rats show most intense uptake in the striatum (Table 1). In the thalamic area, the amount of uptake was much lower than in the striatum, whereas the uptake was lowest in the cerebellum. Total [<sup>123</sup>I]β-CIT uptake was significantly reduced by 56% in the thalamus ( $p < 0.001$ ) and by 32% in the cerebellum ( $p = 0.026$ ), whereas total striatal uptake did not change significantly (-11%,  $p = 0.421$ ). Ratios of [<sup>123</sup>I]β-CIT uptake in the ROI over uptake in the cerebellum were significantly reduced by 37% in the thalamus ( $p = 0.004$ ), but not significantly increased by 28% in the striatum ( $p = 0.065$ ).

## DISCUSSION

Results of both *in vivo* [<sup>123</sup>I]β-CIT SPECT studies, as well as the *ex vivo* biodistribution studies, show that treatment of rats with neurotoxic doses of MDMA lead to significant reductions in the SERT-rich thalamic region. This confirms and extends previous *in vitro*, *ex vivo* and *in vivo* studies in which [<sup>123</sup>I]β-CIT was found to be able to adequately detect 5-HT lesions<sup>3-5</sup>. The observed reduction in thalamic [<sup>123</sup>I]β-CIT SPECT binding ratios, without significant change in striatal binding ratios, likely reflects serotonergic and not dopaminergic neurotoxicity, because it is generally agreed that [<sup>123</sup>I]β-CIT predominantly labels SERT in the midbrain/thalamus, while DAT in the striatum. Displacement studies in humans and monkeys with [<sup>123</sup>I]β-CIT<sup>15,16</sup> and the PET analogue [<sup>11</sup>C]β-CIT<sup>17</sup> showed that specific DAT inhibitors displaced β-CIT in the striatum but not in the midbrain, whereas specific SERT inhibitors displaced β-CIT in the midbrain, but not in the striatum. Moreover, other studies have documented reductions in SERT densities, in animals with known MDMA-induced 5-HT injury, while leaving DAT densities unaffected<sup>2,18</sup>. In mice, serotonin neurons are spared and dopamine neurons are affected after MDMA administration, possibly caused by species dependent mechanisms<sup>19</sup>. Only one recent study reported dopaminergic neurotoxicity in primates, probably related to the recreational dose regimen of repeating administration of relatively small doses of MDMA in short time intervals<sup>20</sup>.

Reneman *et al.* have previously shown thalamic [<sup>123</sup>I]β-CIT binding ratios to be reduced by 59% in *ex vivo* biodistribution studies in MDMA-treated rats, while in the present study thalamic binding ratios were reduced by 37% in *ex vivo*, and by 21% in *in vivo* SPECT studies<sup>5</sup>. This difference in *ex vivo* binding ratios between the two studies is probably related to the lower doses of MDMA administered in the present study. *Ex vivo* binding ratios for thalamus and striatum were much higher than ratios

measured *in vivo*. This is likely to be caused by the partial volume problem induced by measuring radioactivity in the small volume of the thalamus and striatum of rats <sup>21</sup>.

In control rats, the biodistribution study showed most intense uptake of radioactivity in the striatum, with much lower uptake in the thalamus. In the cerebellum, the uptake was lowest, even approximately 5% of striatal uptake. Such a pattern of uptake is in line with previous characterization studies of [<sup>123</sup>I]β-CIT <sup>15</sup>. Due to the extreme low uptake of β-CIT in the cerebellum, most [<sup>123</sup>I]β-CIT SPECT studies have used the cerebellum as a region representing non-specific binding. Interestingly, the present biodistribution study shows that, although the total cerebellar [<sup>123</sup>I]β-CIT binding was low in control rats, it was significantly reduced in MDMA-treated rats. There is evidence that the rat cerebellum is innervated by 5-HT axons and that it therefore contains SERT, although in a low concentration <sup>22</sup>. A recent PET study showed small but significant specific binding of radiotracers that binds to SERTs in monkey cerebellum <sup>23</sup>. Since cerebellar [<sup>123</sup>I]β-CIT uptake in human studies is generally used as a reference for non-specific uptake, and binding ratios are expressed as total binding divided by cerebellar binding, our present finding may indicate, if replicated in human brain, that reductions in binding ratios underestimate reductions in SERT and consequently neurotoxicity. However, since this study is performed in small groups of animals and the cerebellar uptake of [<sup>123</sup>I]β-CIT is extreme low and therefore prone to low reproducibility, our relevant finding of reduced [<sup>123</sup>I]β-CIT uptake in the cerebellum needs to be confirmed in larger studies.

The present observation that [<sup>123</sup>I]β-CIT pinhole SPECT may be useful in studying *in vivo* MDMA-induced neurotoxic changes is of interest for a number of reasons. It enables to perform serial studies in MDMA-treated small animals in an experimental setting, without the need of sacrificing the animals after each experiment. It may therefore be suitable especially for examining potential confounders that are almost impossible to study experimentally in humans, like effects of temperature and (de)hydration, dosing patterns, age, the effects of combined use of other drugs of abuse on the neurotoxic action of MDMA and the effects of potential neuroprotective agents like selective serotonin reuptake inhibitors and anti-oxidants. Besides, it may be possible to study long-term effects of MDMA administration on cognitive functioning <sup>24</sup> in older animals in a more accurate way, linking it to temporal changes in SERT and/or DAT densities.

Additionally it may become possible to study *in vivo* in an experimental setting other diseases with known involvement of the serotonergic system, like depression and anxiety disorders.

Finally, with optimisation of the co-registration of MR with SPECT images by using external radioactive markers <sup>10</sup>, it will become possible in future studies to examine also brain regions containing low concentrations of SERTs, like the cerebral cortex and hippocampus.

In conclusion, our preliminary *ex vivo* and *in vivo* results provide evidence that it is possible to detect MDMA-induced loss of SERTs in the living rat brain by using [<sup>123</sup>I]β-CIT SPECT. The newly developed high-resolution pinhole SPECT technique may be a promising technique for performing serial studies of MDMA-induced serotonergic neurotoxicity, and other diseases involving the serotonergic system, in living small animals.

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CHAPTER  
5

**Validation of [<sup>123</sup>I]β-CIT SPECT to Assess Serotonin Transporters *In Vivo* in Humans: a Double-Blind, Placebo-Controlled, Crossover Study with the Selective Serotonin Reuptake Inhibitor Citalopram**

Neuropsychopharmacology 2005; 30 : 996-1005

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# ABSTRACT

## Background

Disturbances in the serotonin (5-HT) system are associated with various neuropsychiatric disorders. The 5-HT system can be studied *in vivo* by measuring 5-HT transporter (SERT) densities using <sup>123</sup>Iodine-labeled 2β-carbomethoxy-3β(4-iodophenyl)tropane ([<sup>123</sup>I]β-CIT) and single photon emission computed tomography (SPECT). Validation of this technique is important because [<sup>123</sup>I]β-CIT does not bind selectively to SERTs. Some studies have validated this technique *in vivo* in the human brain in SERT-rich areas, but the technique has not been validated yet in SERT-low cortical areas.

## Aim

To further validate [<sup>123</sup>I]β-CIT SPECT in assessing SERTs *in vivo* in humans in both SERT-rich and SERT-low areas.

## Material and Methods

A double-blind, placebo-controlled, crossover design was used with the selective serotonin reuptake inhibitor (SSRI) citalopram. Six male subjects underwent two [<sup>123</sup>I]β-CIT SPECT sessions: one after pretreatment with citalopram and one after placebo. Scans were acquired 4 h and 22-27 h p.i., and both region-of-interest and voxel-by-voxel analyses were performed.

## Results

Citalopram reduced [<sup>123</sup>I]β-CIT binding ratios in SERT-rich midbrain and (hypo)thalamus. Binding ratios were also lower after citalopram in SERT-low cortical areas, but statistical significance was only reached in several cortical areas using voxel-by-voxel analysis. In addition, citalopram increased binding ratios in the DAT-rich striatum and increased absolute uptake in the cerebellum.

## Conclusions

The results show that [<sup>123</sup>I]β-CIT SPECT is a valid technique to study SERT binding *in vivo* in human brain in SERT-rich areas. Although we provide some evidence that [<sup>123</sup>I]β-CIT SPECT may be used to measure SERTs in SERT-low cortical areas, these measurements must be interpreted with caution.

## INTRODUCTION

Nowadays, it is possible to study the serotonergic system in the living human brain by visualizing the serotonin (5-HT) transporter (SERT) at the terminus of the serotonergic axon. The SERT is a plasma membrane and a structural element of the pre-synaptic membrane of the serotonergic axon. Therefore, it is used as a reliable marker for the serotonergic axon<sup>1</sup>. SERT density can be assessed using a radioligand that binds to SERT in combination with positron emission tomography (PET) or single photon emission computed tomography (SPECT).

The serotonergic system modulates many neuropsychological processes, such as mood and memory, through the neurotransmitter serotonin. Disturbances in the serotonergic system are associated with neuropsychiatric disorders including depression, eating disorders, and Alzheimer's disease<sup>2,3</sup>. There is also evidence that the popular recreational drug ecstasy (3,4-methylenedioxymethamphetamine, MDMA) causes damage to the serotonergic axons both in animals<sup>4,5</sup> and humans<sup>6,7</sup>. The use of ecstasy is associated with decreased memory function and increased symptoms of depression<sup>8-10</sup>. SERT is also the primary site of action for selective serotonin reuptake inhibitors (SSRIs), the most frequently prescribed anti-depressants. Therefore, visualization and quantification of SERT is important for studying neuropsychiatric disorders in which the serotonergic system is involved and for studying occupancy of SERTs by SSRIs *in vivo*.

One of the SPECT ligands that is used for studying SERTs is  $^{123}$ iodine-labeled 2 $\beta$ -carbomethoxy-3 $\beta$ (4-iodophenyl)tropane ([ $^{123}$ I] $\beta$ -CIT), a cocaine derivate that binds with high affinity to SERTs and dopamine transporters (DATs)<sup>11</sup>. The use of [ $^{123}/^{125}$ I] $\beta$ -CIT in visualizing SERTs and DATs has been validated *in vitro* and *ex vivo* in rodents<sup>1,12-14</sup> and in human brain slices<sup>15</sup>. In addition, *in vivo* displacement studies in monkeys demonstrated that binding in the hypothalamus and midbrain was mainly associated with binding to SERTs, while binding in the striatum was mainly associated with binding to DATs<sup>11</sup>. Therefore, it may be possible with [ $^{123}$ I] $\beta$ -CIT SPECT to study both SERT and DAT densities in anatomical separated areas.

In humans, *in vivo* [ $^{123}$ I] $\beta$ -CIT SPECT imaging of the SERT has been used, among others, to study SERT availability in depression and alcoholism and to study the neurotoxic effects of ecstasy<sup>16-19</sup>. However, because [ $^{123}$ I] $\beta$ -CIT does not bind selectively to SERTs, but also to DATs and norepinephrine transporters, there are some difficulties in using [ $^{123}$ I] $\beta$ -CIT SPECT for studying SERT binding *in vivo*. Therefore, it is important to validate in which brain areas [ $^{123}$ I] $\beta$ -CIT can be accurately used to study SERTs. Although several studies used [ $^{123}$ I] $\beta$ -CIT SPECT to assess SERTs *in vivo* in humans, validation of this technique has been limited.

First, a limited number of studies have been performed to validate the use of [ $^{123}$ I] $\beta$ -CIT SPECT for studying SERTs *in vivo* in humans<sup>20,21</sup> or to study the effects

of [ $^{123}\text{I}$ ] $\beta$ -CIT binding to SERTs and DATs *in vivo* in humans after blocking of SERTs with a SSRI <sup>20,22,23</sup>. One study validated the use of *in vivo* [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT in detecting MDMA-induced serotonergic neurotoxicity in SERT-rich brain areas in a monkey <sup>14</sup>.

Second, previous SSRI displacement studies did not validate the *in vivo* [ $^{123}\text{I}$ ] $\beta$ -CIT binding to SERTs in cortical regions, probably because the interpretation in these areas is hampered by low SERT and DAT densities <sup>24-26</sup>. Since studies in non-human primates showed long-term and possible irreversible damage of serotonergic axons in the cerebral cortex induced by MDMA <sup>4</sup>, assessment of SERT density in the cerebral cortex is especially important for studying the neurotoxic effects of ecstasy. Reductions of SERT densities in cortical brain regions were reported in male <sup>27</sup> and female <sup>18</sup> ecstasy users. However, others discussed these results because of the presumed limited sensitivity of [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT imaging to measure SERT density in the cerebral cortex <sup>26,28,29</sup>.

Third, previous validation studies have been limited by some methodological weaknesses. Some of these studies were performed in depressed patients, whereas depression may be an important confounder. For example, lower [ $^{123}\text{I}$ ] $\beta$ -CIT binding ratios were reported in SERT-rich areas in depressed patients treated with SSRIs compared to healthy volunteers <sup>20,23</sup>, whereas also in untreated depressive patients decreased SERT binding has been described <sup>17</sup>. Only Kugaya and co-workers studied the *in vivo* alterations of [ $^{123}\text{I}$ ] $\beta$ -CIT binding during SSRI treatment in both depressive patients and in healthy volunteers <sup>22</sup>. To our knowledge, no validation studies have been performed yet in the same healthy subjects before and after SSRI treatment.

The purpose of the present study was to further validate the technique of [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT to assess SERTs *in vivo* in humans in both SERT-rich and SERT-low brain areas using the most optimal study design. Therefore, a double-blind, placebo-controlled, crossover design with the most selective SSRI citalopram was used to study the binding of [ $^{123}\text{I}$ ] $\beta$ -CIT to SERTs and DATs in SERT-rich, DAT-rich and in SERT-low cortical brain regions. As citalopram selectively blocks SERTs, we hypothesized that [ $^{123}\text{I}$ ] $\beta$ -CIT binding ratios would decrease in both SERT-rich areas and in SERT-low cortical areas after pre-treatment with citalopram.

## MATERIAL AND METHODS

### Subjects

Six healthy male volunteers were included in the present study between November 2002 and May 2003. They were recruited by means of posters at the Faculty of Medicine of the University of Amsterdam, The Netherlands. Exclusion criteria were age below 18

or above 35 years, major mental or physical problems, use of psychopharmaceuticals such as SSRIs, and use of hard drugs such as ecstasy, cocaine, amphetamine, or heroine in the past. All volunteers had to complete the Beck Depression Inventory (BDI<sup>30</sup>), a validated depression questionnaire, before each imaging session to exclude depression. Participant with a BDI  $\geq 10$  were excluded. Written informed consent was obtained from all subjects and the study was approved by the local medical ethics committee.

## Study-procedure

We used a double-blind, placebo-controlled, crossover design to study the effect of the SSRI citalopram on binding of [<sup>123</sup>I]β-CIT to SERTs and DATs. Therefore, all six subjects participated in two different sessions with an interval of 4-6 weeks between both sessions. At 3 h before injection with [<sup>123</sup>I]β-CIT, the subjects were given a capsule with either 20 mg of the most selective SSRI citalopram<sup>31</sup> or a placebo that had to be taken orally under supervision of the examiner. Both volunteer and examiner were blinded for the content of the capsule. At 3 h after intake of the capsule, [<sup>123</sup>I]β-CIT was injected. Per session two SPECT scans were acquired, 4 h post-injection (p.i.) as well as 22-27 h p.i., when stable uptake to the SERTs and DATs is expected to be reached, respectively<sup>21,32</sup>. After 4-6 weeks the procedure was repeated and subjects who were given citalopram the first session got placebo at the second session, and *vice versa*.

## [<sup>123</sup>I]β-CIT SPECT imaging

Subjects were examined using SPECT with the radioligand [<sup>123</sup>I]β-CIT that binds to SERTs, DATs, and, in a lesser extent, also to norepinephrine transporters. Radiosynthesis of [<sup>123</sup>I]β-CIT was performed by electrophilic radioiododestannylation of the trimethyltin precursor (RadioNuclide Center, Vrije Universiteit Amsterdam, The Netherlands), with carrier-free [<sup>123</sup>I] as NaI (BV Cyclotron, Vrije Universiteit) in the presence of peracetic acid as oxidant. After labeling, the product was further purified with HPLC separation, diluted and sterile filtrated to an injectable solution with a radioactive concentration of 55-60 MBq/ml. The radiochemical purity of the solution was higher than 99.5 %. At both sessions, 112 MBq (3.02 mCi) [<sup>123</sup>I]β-CIT was injected intravenously as a bolus. Subjects received a potassium iodide solution to block thyroid uptake of free radioactive iodide.

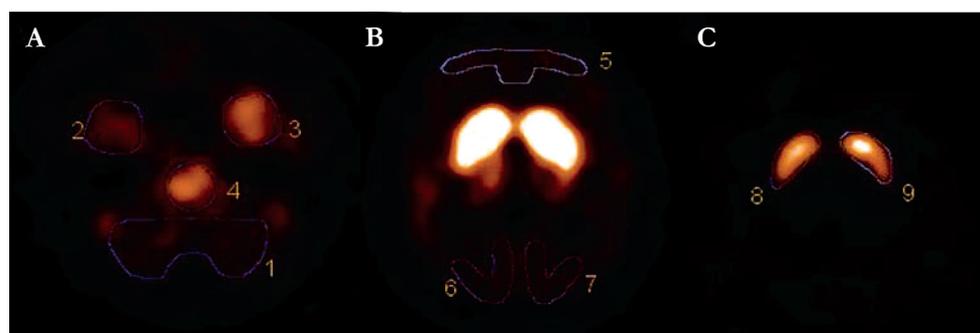
SPECT studies were performed using a 12-detector single slice brain-dedicated scanner (Neurofocus 810, which is an upgrade of the Strichmann Medical Equipment) with a full-width at half-maximum resolution of approximately 6.5 mm, throughout the 20 cm field-of-view (<http://www.neurophysics.com>). After positioning of the

subjects with the head parallel to the orbitomeatal line, axial slices parallel and upward from the orbitomeatal line to the vertex were acquired in 5 mm steps. Each acquisition consisted of approximately 15 slices with 3 min scanning time per slice, acquired in a 64 x 64 matrix. The energy window was set at 135-190 keV.

## Image Reconstruction and Analysis

Attenuation correction of all images was performed as described earlier<sup>33</sup>. Images were reconstructed in 3D mode (<http://www.neurophysics.com>). For quantification, both region-of-interest (ROI) and voxel-by-voxel analyses were performed.

Standardized templates of 2D ROIs were drawn with the help of a high-resolution MRI and a brain atlas. For the SPECT scans 4 h p.i., ROIs for midbrain, (hypo)thalamus, frontal cortex, temporal cortex, occipital cortex, and cerebellum were used. For the SPECT scan 22-27 h p.i., ROIs for the striatum and cerebellum were used. The ROIs were positioned on the SPECT slices by the same examiner who was blinded for the content of the pretreatment (Figure 1). For the right and left striatum, a template with irregular ROIs, according to the contour of the putamen and caudate nucleus, was positioned on four consecutive axial slices with highest striatal activity. Individual variation required movement of the fixed ROIs, without changing size and shape, within the template for optimal fitting. For the frontal, right occipital, and left occipital cortices, irregular ROIs were drawn in one template. The template was positioned on four consecutive slices: on the three superior slices where the striatal ROIs were positioned and on one slice above. We defined the inferior level of the (hypo)thalamus to correspond with the inferior level of the striatum. For the (hypo)thalamic area, a template with an oval-shaped ROI was placed on four SPECT



**Figure 1:** [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT images with ROI templates of cerebellum (1), right temporal cortex (2), left temporal cortex (3), midbrain (4), frontal cortex (5), right occipital cortex (6), left occipital cortex (7), right striatum (8) and left striatum (9). Images A and B are 4 h p.i. and image C is 24 h p.i. The level of radioactivity is colour encoded from low (black) through medium (yellow) to high (white) and scaled to the maximum of the study. To visualize low specific binding to SERTs in images A and B, the upper threshold was set at approximately 25% of the maximum of the study. (see page 309 for colour illustration)

slices with the highest (hypo)thalamic activity. The superior level of the midbrain was defined to coincide with the most superior slice without visible striatal activity. For this midbrain area, a template with a round-shaped ROI was placed on four SPECT slices with the highest activity of the midbrain. For the cerebellum, a template with an irregular shaped ROI was positioned on three SPECT slices with highest cerebellar activity. A template with irregular ROIs for right and left temporal cortex was placed on the two most superior slices where the cerebellum template was positioned. Mean striatal, mean temporal, and mean occipital binding densities were averaged from right and left ROIs. Mean cortical binding was calculated as the mean counts per voxel from the frontal, temporal, and occipital cortex together. Absolute uptake in the cerebellum was defined as the mean activity within the ROI of the cerebellum divided by the injected dose (Bq/ml/MBq). Activity in the cerebellum was assumed to represent nondisplaceable activity (nonspecific binding and free radioactivity). Specific to nonspecific binding ratios were calculated as (activity in ROI – activity in cerebellum)/ activity in cerebellum.

For the voxel-by-voxel analysis, Statistical Parametric Mapping software (SPM 99, welcome of Cognitive Neurology, Institute of Neurology, London, UK; [www.fil.ion.ucl.ac.uk/spm](http://www.fil.ion.ucl.ac.uk/spm)) implemented in matlab (Mathworks, Sherborn, MA) was used<sup>34</sup>. All SPECT images were spatially normalized by a linear coregistration to a standard stereotaxic space<sup>35</sup>. Subsequently, the images were intensity-scaled to the corresponding mean cerebellar nonspecific counts per voxel. The mean cerebellar counts were obtained from the ROI analysis.

## Statistical analysis

Differences in binding densities in ROIs between citalopram and placebo pretreatment were analyzed using a two-sided Student's *t*-test for paired samples. The Kolmogorov-Smirnov test was used to analyze whether data were normally distributed. Statistical significance was defined as  $p < 0.05$ . Statistical analyses were performed using SPSS 11.5.

Based on the coregistered images, statistical parametric maps of the paired *t*-statistics SPM{T} were calculated and transformed to the unit normal distribution SPM{Z}. Only clusters of connected voxels above an extent threshold of 20 voxels (voxel threshold was set at  $p < 0.01$ , Z-score = 2.33, uncorrected for multiple comparisons) were tested for significance by means of spatial extent statistical theory<sup>36,37</sup>. Clusters of voxels surviving the thresholds were color-coded and superimposed on reference images.

**Table 1:** Results of the ROI analysis of the *in vivo* binding of [<sup>123</sup>I]β-CIT in midbrain, (hypo)thalamus, striatum and in cortex (temporal, frontal, occipital and mean cortex). [<sup>123</sup>I]β-CIT

		Cerebellum 4 h p.i.	
		Placebo	Citalopram
Absolute uptake <sup>†</sup> [Bq/ml/MBq]	subject 1	20.27	24.16
	subject 2	17.29	20.82
	subject 3	15.11	22.44
	subject 4	14.55	19.40
	subject 5	18.19	17.36
	subject 6	22.79	26.96
	<b>mean</b>	<b>18.03 ± 3.13</b>	<b>21.85 ± 3.44*</b>
		Midbrain	
		Placebo	Citalopram
Ratios <sup>‡</sup>	subject 1	0.54	0.27
	subject 2	0.70	0.47
	subject 3	0.62	-0.00
	subject 4	1.08	0.07
	subject 5	0.72	0.42
	subject 6	1.05	0.23
	<b>mean</b>	<b>0.88 ± 0.23</b>	<b>0.24 ± 0.19*</b>
		Temporal cortex	
		Placebo	Citalopram
Ratios <sup>‡</sup>	subject 1	0.28	0.19
	subject 2	0.29	0.11
	subject 3	0.39	-0.23
	subject 4	0.22	-0.01
	subject 5	0.34	0.40
	subject 6	0.22	-0.08
	<b>mean</b>	<b>0.29 ± 0.07</b>	<b>0.06 ± 0.22</b>

\* uptake significant different from placebo (paired Student *t*-test), *p* < 0.05

† absolute uptake, expressed as mean uptake (Bq) per ml within the ROI, divided by the injected dose in MBq

## RESULTS

Mean age of the subjects was 24.0 ± 4.3 years (range 18.7 - 31.2 years). Mean BDI scores were 1.3 ± 2.3 (range 0-6) at placebo session and 1.3 ± 1.9 (range 0-5) at citalopram session. As BDI scores between 0 and 9 are regarded within the normal range, none of the subjects had clinical depression.

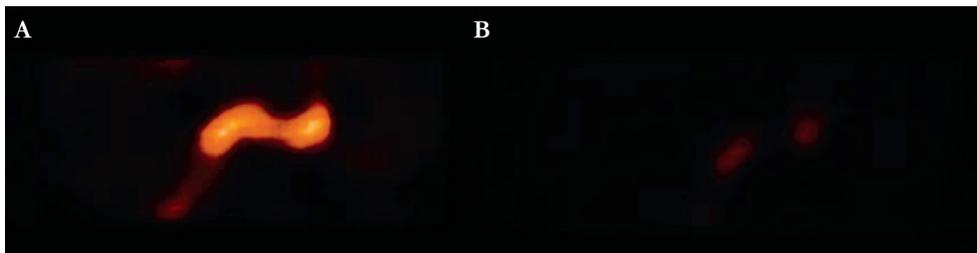
## ROI analysis

Data from the ROI analysis were normally distributed. The individual and mean absolute uptake in the cerebellum and the individual and mean uptake ratios within ROIs are described in Table 1. Figure 2 shows a representative sagittal slice of

uptake was measured 4 h p.i., except for striatal uptake which was measured 24 h p.i., and cerebellar uptake which was measured 4 and 24 h p.i.

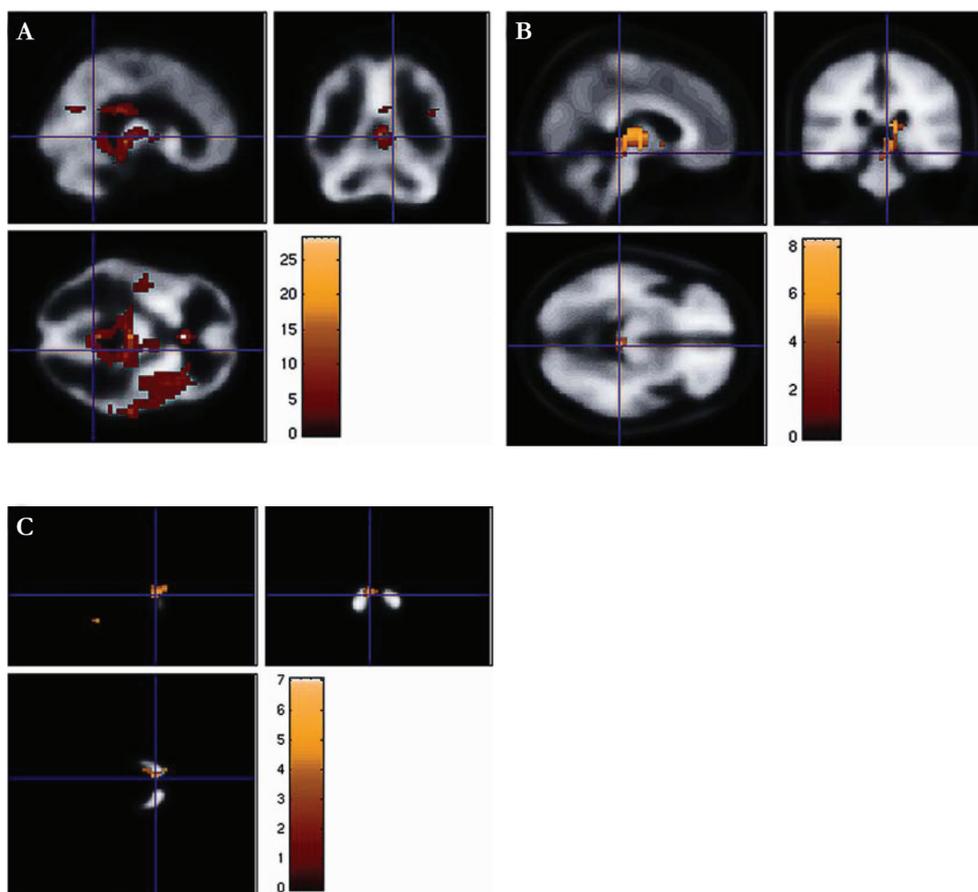
Cerebellum 24 h p.i.					
Placebo		Citalopram			
4.10		5.87			
5.32		3.83			
3.33		4.39			
4.92		3.75			
5.17		3.34			
5.18		3.83			
<b>4.67 ± 0.79</b>		<b>4.17 ± 0.90</b>			
(Hypo)thalamus		Striatum (24 h p.i.)			
Placebo		Citalopram		Placebo	
				Citalopram	
1.15		0.56		9.53	
1.31		0.45		9.23	
1.35		0.34		9.56	
1.70		0.64		9.40	
1.29		0.87		9.93	
1.15		0.44		9.60	
<b>1.32 ± 0.20</b>		<b>0.55 ± 0.19*</b>		<b>9.54 ± 0.23</b>	
				<b>12.04 ± 1.41*</b>	
Frontal cortex		Occipital cortex		Mean cortex	
Placebo		Citalopram		Placebo	
				Citalopram	
-0.01		-0.05		-0.06	
0.15		-0.10		-0.03	
0.10		-0.19		0.07	
0.17		-0.19		0.01	
-0.06		0.10		0.12	
0.10		-0.17		-0.05	
<b>0.07 ± 0.09</b>		<b>-0.10 ± 0.11</b>		<b>0.02 ± 0.06</b>	
				<b>-0.10 ± 0.11</b>	

‡ uptake ratios expressed as [uptake in the region of interest minus uptake in cerebellum] divided by uptake in the cerebellum



**Figure 2:** Representative sagittal images of the [<sup>123</sup>I]β-CIT uptake in the midline 4 h p.i. after placebo pretreatment (A) and after citalopram pretreatment (B). The level of radioactivity is colour encoded from low (black) through medium (yellow) to high (white) and scaled to the maximum of the study. (☞ page 309 for colour illustration)

the activity uptake in the midline 4 h p.i. after placebo pretreatment (A) and after citalopram pretreatment (B). Absolute nonspecific cerebellar activity was significantly higher after citalopram pretreatment than after placebo intake 4 h, but not 24 h p.i. Binding ratios of [ $^{123}\text{I}$ ] $\beta$ -CIT 4 h p.i. were significantly lower after citalopram intake than after placebo intake in the SERT-rich midbrain ( $p = 0.009$ ) and (hypo)thalamus ( $p = 0.001$ ). [ $^{123}\text{I}$ ] $\beta$ -CIT binding ratios 4 h p.i. were also lower after citalopram intake than after placebo intake in the SERT-low temporal, frontal, and occipital cortical areas, although these findings did not reach statistical significance ( $p = 0.055$ ,  $p = 0.088$ , and  $p = 0.340$ , respectively). The calculated mean cortical binding ratio was



**Figure 3:** Clusters of significant different binding ratios after voxel-by-voxel analysis with citalopram pretreatment compared to placebo pretreatment, superimposed on reference images. Image **A** shows 4 h p.i. lower binding ratios in subcortical (insula, midbrain and thalamus) and in several cortical areas (e.g. middle- and superior temporal gyrus, inferior-, middle -and superior frontal gyrus, anterior cingulate, and postcentral gyrus). The clusters high on the sagittal slice are related to the upper boundary of the field of view and are likely to represent artifacts rather than real significant differences in [ $^{123}\text{I}$ ] $\beta$ -CIT binding ratios. Image **B** shows lower binding ratios in the midbrain/ (hypo)thalamus area and image **C** shows increased binding ratio in the left caudate nucleus 22-27 h p.i. (see page 310 for colour illustration)

also not significantly different between the two sessions ( $p = 0.124$ ). The binding ratio in the DAT-rich striatum 22-27 h p.i. was significantly higher ( $p = 0.009$ ) after citalopram intake than after placebo intake.

## Voxel-by-voxel analysis

Voxel-by-voxel analysis of the scans made 4 h p.i. showed clusters of significantly lower [<sup>123</sup>I]β-CIT binding ratios in the SERT-rich midbrain and thalamus ( $Z_{\max} = 4.45$ ,  $p_{\text{cluster level}} < 0.001$ ) after pretreatment with citalopram. Clusters of significantly decreased binding ratios after citalopram pretreatment were also found in several SERT-low (mainly) cortical areas, for example, subcortical insula, left and right middle- and superior temporal gyrus, inferior frontal gyrus, and postcentral gyrus ( $Z_{\max} = 4.20$ ,  $p_{\text{cluster level}} < 0.001$ ), right middle -and superior frontal gyrus ( $Z_{\max} = 3.74$ ,  $p_{\text{cluster level}} = 0.047$ ) and anterior cingulate ( $Z_{\max} = 4.88$ ,  $p_{\text{cluster level}} = 0.004$ ) (Figure 3a). Analysis of the scans 22-27 h p.i. showed a cluster of significantly lower binding ratios in the midbrain/thalamus area ( $Z_{\max} = 3.53$ ,  $p_{\text{cluster level}} < 0.001$ ) after citalopram pretreatment (Figure 3b) and increased binding ratios in the DAT-rich left caudate nucleus ( $Z_{\max} = 3.32$ ,  $p_{\text{cluster level}} < 0.001$ ) (Figure 3c).

## DISCUSSION

To our knowledge, this is the first study that validated the use of [<sup>123</sup>I]β-CIT SPECT to assess SERTs *in vivo* in the human brain using a double-blind and placebo-controlled design in which the same healthy volunteers were examined with and without pretreatment with the most selective SSRI citalopram. The potential confounders age, gender, and depression were excluded because we only included men within a small age-range without depression. Although [<sup>123</sup>I]β-CIT SPECT is currently not used as a diagnostic tool to assess SERT densities, validation of this technique is important because a valid technique would make it possible to adequately study the serotonergic system *in vivo* in several diseases such as mood disorders, Parkinson's disease, eating disorders, alcoholism, and MDMA neurotoxicity. Moreover, [<sup>123</sup>I]β-CIT SPECT could be used to evaluate the occupancy of SERTs by medication such as SSRIs.

In the present study, we measured (with ROI analyses) [<sup>123</sup>I]β-CIT uptake ratios for SERT binding 4 h p.i., although kinetic studies showed that in SERT-rich areas binding ratios were closer to a state of transient equilibrium between 20 and 24 h p.i. than 4 h p.i. (increase in binding ratios in the midbrain of  $5.5\% \pm 3.9\%/h$  vs  $2.5\% \pm 5.3\%/h$  at 4-10 h p.i. and 20-24 h p.i., respectively)<sup>21</sup>. As the differences were small and because several studies reported stable uptake in (hypo)thalamus, midbrain, cerebral cortex, and cerebellum at 4 h p.i.<sup>20,38</sup>, it was suggested that both time points

are reliably for measurements of SERTs with [ $^{123}\text{I}$ ] $\beta$ -CIT<sup>21</sup>. Moreover, as the count statistics are better 4 h than 24 h p.i., leading to better visibility especially of the SERT-low cortical areas, it would be likely that measurements of SERT binding 4 h p.i. have better test-retest reliability than measurements of SERT binding 24 h p.i., although we did not study this.

The most important results of our study are that [ $^{123}\text{I}$ ] $\beta$ -CIT binding ratios were lower in SERT-rich as well as in SERT-low areas (although only statistically significant with voxel-by-voxel analysis and not with ROI analysis) and higher in the DAT-rich striatum after pre-treatment with citalopram compared to placebo. Moreover, we found that citalopram increased the absolute [ $^{123}\text{I}$ ] $\beta$ -CIT in the SERT-low and DAT-low cerebellum. These results will be discussed in detail in the following paragraphs.

First, in the SERT-rich midbrain and (hypo)thalamus, [ $^{123}\text{I}$ ] $\beta$ -CIT binding significantly decreased with 72 and 58%, respectively (ROI analysis), after pretreatment with citalopram. This corroborates previous findings of *in vivo* studies in humans, which showed that citalopram significantly inhibited [ $^{123}\text{I}$ ] $\beta$ -CIT binding to SERTs in the brainstem and diencephalon<sup>20,22</sup>. Human PET studies using the SERT-selective radiotracer [ $^{11}\text{C}$ ]DASB also showed high occupancy of SERTs in the midbrain and thalamus after (sub)therapeutic doses of various SSRIs<sup>39</sup>. In addition, previous studies in animals showed that [ $^{123}\text{I}$ ] $\beta$ -CIT binds to SERTs in these brain areas<sup>11</sup>. Decreased binding of [ $^{123}\text{I}$ ] $\beta$ -CIT to SERTs after pretreatment with citalopram could be caused by decreased availability of SERTs due to blockage of SERTs by citalopram, by down-regulation of the SERT protein, by a change in affinity of [ $^{123}\text{I}$ ] $\beta$ -CIT for the SERT, or by a combination of these factors. Benmansour *et al*<sup>40</sup> showed that marked loss of SERT binding sites due to down-regulation occurred in rats only after 10-15 days of SSRI treatment. Therefore, we think it is not likely that SERT down-regulation in our subjects occurred within one day after only one dose of citalopram. However, down-regulation of SERT proteins may play a role in subjects who have taken SSRI treatment for a longer period. Although we cannot exclude that SSRIs induce fast changes in the affinity of [ $^{123}\text{I}$ ] $\beta$ -CIT for SERTs, the presently observed decreased binding of [ $^{123}\text{I}$ ] $\beta$ -CIT in SERT-rich brain areas after citalopram pretreatment is most likely explained by acute blockage of SERT binding sites.

Second, besides a decrease in [ $^{123}\text{I}$ ] $\beta$ -CIT binding in SERT-rich areas, we also found a decrease in [ $^{123}\text{I}$ ] $\beta$ -CIT binding ratios in SERT-low cortical areas after pretreatment with citalopram. Voxel-by-voxel analysis showed clusters of significant decreases within several cortical areas. Using ROI analysis, the decreases in cortical binding did not reach the level of statistical significance, possibly because of low statistical power. Reduction of [ $^{123}\text{I}$ ] $\beta$ -CIT binding after SSRI-pretreatment has also been shown in the prefrontal cortex of rats<sup>1</sup>. Moreover, autoradiographic studies of human brain sections showed that citalopram, but not the selective norepinephrine re-uptake blocker desipramine or the selective dopamine transporter blocker GBR12909, was capable to displace cortical

binding of the PET ligand [ $^{11}\text{C}$ ] $\beta$ -CIT<sup>41</sup>. In addition, significant reductions of [ $^{123}\text{I}$ ] $\beta$ -CIT binding ratios were observed in various cortical areas of rats after pretreatment with MDMA, which is considered to be a selective neurotoxin for serotonergic neurons<sup>14</sup>. In line with this, [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT studies in human ecstasy users showed reductions of SERT in cortical brain regions<sup>18,19,27</sup>. These findings suggest that [ $^{123}\text{I}$ ] $\beta$ -CIT binding in the cerebral cortex is predominantly caused by binding to SERTs. However, other studies were not able to show that [ $^{123}\text{I}$ ] $\beta$ -CIT binds to cortical SERTs<sup>11</sup> or did not study this issue<sup>20,22</sup>. In addition, a SSRI displacement study with the SERT-selective PET radioligand [ $^{11}\text{C}$ ]McN 5652 in non-human primates also failed to show specific binding in the cerebral cortex<sup>42</sup>. Finally, studies showed in rats<sup>1</sup> and in postmortem human brains<sup>43</sup> that cortical [ $^{123}\text{I}$ ] $\beta$ -CIT binding was mainly blocked by citalopram, and to a lesser extent also by selective DAT and norepinephrine reuptake inhibitors. Therefore, using the radiotracer [ $^{123}\text{I}$ ] $\beta$ -CIT it will be very difficult to distinguish between SERT and DAT binding in the cortical areas and this would only be possible when selective blockers for DAT and norepinephrine transporter are used simultaneously. As expected, we found much lower [ $^{123}\text{I}$ ] $\beta$ -CIT binding ratios in the cortex than in the midbrain and (hypo)thalamus, because the SERT concentration is much lower in the cerebral cortex than in the midbrain or diencephalon<sup>24,25</sup>. These low binding densities and corresponding low signal-to-noise ratios cause poor visualization and delineation by which placement of ROI templates in the cerebral cortex is hampered. Owing to these arguments, we think that measurement of SERT in the cortical areas using [ $^{123}\text{I}$ ] $\beta$ -CIT must be interpreted with caution, although SPM data provide evidence that [ $^{123}\text{I}$ ] $\beta$ -CIT most likely binds to SERTs in specific SERT-low brain regions such as the temporal and frontal cortex. More selective SERT ligands for SPECT imaging, such as the recently developed [ $^{123}\text{I}$ ]ADAM with a 1000-fold greater selectivity to SERTs than to norepinephrine transporters or DATs<sup>44</sup>, will probably be more sensitive to the detection of decreased SERT availability in the cortical areas.

Third, in contrast to a decreased binding to SERTs we observed an increased binding of [ $^{123}\text{I}$ ] $\beta$ -CIT in the DAT-rich striatum 22-27 h p.i. after pretreatment with citalopram compared to placebo. From other studies, it is known that SERTs are highly expressed in human lungs and that SSRIs accumulate in here<sup>45</sup>. Additionally, human biodistribution studies with [ $^{123}\text{I}$ ] $\beta$ -CIT showed intense binding in the lungs, liver and lower large intestine<sup>46</sup>. It is therefore likely that peripheral blockage of SERTs by citalopram has resulted in increased tracer availability in the brain. However, kinetic studies showed that the specific binding of [ $^{123}\text{I}$ ] $\beta$ -CIT to DATs in human brain is stable 20-30 h p.i. and the kinetic model predicts that both specific and nonspecific binding would increase in the same proportion<sup>32</sup>. Therefore, it is not expected that a change in radiotracer availability would influence the striatal binding ratio. On the other hand, the finding of increased striatal activity after administration of SSRIs was

also observed in several other studies in animals<sup>47,48</sup> and in humans<sup>22,23</sup>. Kugaya *et al* suggested that the increase might be caused by interaction between serotonin and dopamine systems<sup>22</sup>. Scheffel *et al* suggested that pretreatment with SSRIs would cause displacement of the radiotracer from peripheral and central SERTs, and that this would lead to an increased availability of radiotracer for binding to DATs<sup>48</sup>. In accordance with Scheffel *et al*<sup>48</sup>, we hypothesize that the combination of increased tracer availability (due to peripheral SERT blockage) and decreased SERT availability (due to central SERT blockage) in the brain might have led to increased availability of the tracer for binding to DATs, consequently leading to the observed increased uptake ratio in the DAT-rich striatum when compared to the DAT-devoid reference area of the cerebellum. Although the underlying mechanism is debatable the finding deserves attention in future studies. This is, for example, of importance because [<sup>123</sup>I]β-CIT SPECT studies are performed frequently to measure striatal DATs in parkinsonian patients, and such patients quite often use SSRIs.

Fourth, we observed an increase of absolute cerebellar binding after pretreatment with citalopram, 4 h p.i. This increased binding was probably also caused by increased availability of radiotracer in the brain. Szabo *et al*<sup>47</sup> reported a strong increase of brain uptake of the selective SERT tracers [<sup>11</sup>C]DASB and [<sup>11</sup>C]McN5652 in baboons treated with the SSRI paroxetine. The authors suggested that this finding was caused by a combination of a decreased peripheral binding to SERTs and a paroxetine-induced reduction of the metabolism of both radiotracers. In our study, we did not measure the influence of citalopram on the metabolism of [<sup>123</sup>I]β-CIT. Although in literature there are no indications that SSRIs influence the metabolism of [<sup>123</sup>I]β-CIT, we cannot exclude this as a cause of enhanced brain uptake. We believe it is more likely, however, that blockage of peripheral SERTs by citalopram has led to an increased absolute brain uptake (including increased nonspecific cerebellar uptake) of [<sup>123</sup>I]β-CIT, although Farde *et al* did not observe a change in cerebellar activity of the PET ligand [<sup>11</sup>C]β-CIT after the administration of Citalopram<sup>41</sup>. This discrepancy emphasizes the need for more studies on the influence of SSRIs on metabolism and on binding of SERT radiotracers to central as well as peripheral SERTs. It is not likely that binding ratios have been affected by the increased cerebellar uptake, because the binding ratios are considered to be stable 4 h p.i.<sup>20,21,38</sup>. Therefore, increased tracer availability would increase both specific and nonspecific (cerebellar) binding in the same proportion. This means that the presently observed lower binding ratios in SERT-rich and cortical areas after citalopram presumably reflect blocking of specific binding to SERTs.

In contrast to the above mentioned increased absolute cerebellar uptake of SERT radiotracers after pretreatment with SSRIs, pretreatment with the selective serotonin neurotoxin MDMA significantly decreased cerebellar uptake of activity in the same study of Szabo *et al* and in a study with rats using the radioligand [<sup>123</sup>I]β-CIT<sup>47,49</sup>. Since

the cerebellum is not devoid of SERTs<sup>24,25</sup>, this might be explained by an MDMA-induced decreased availability of SERTs in the cerebellum. Both SSRI and MDMA pretreatment could be used to evaluate the specificity of radiotracer binding to SERTs. However, the contrasting findings on cerebellar (nonspecific) binding emphasize that results from different studies should be interpreted with caution, because differences in binding may not solely reflect blockage of binding from specific central binding sites. In case of measuring MDMA-induced neurotoxicity, it has been advocated to use specific to nonspecific ratios, with the cerebellar binding as an adequate estimate of nonspecific binding. As specific binding ratios are calculated by the formula [activity in ROI – activity in cerebellum] divided by activity in cerebellum, MDMA-induced loss of cerebellar SERT binding might lead to an overestimation of SERT density and thus an underestimation of the presumably neurotoxic effects of MDMA. Therefore, it has been suggested that the specific to nonspecific SERT binding ratios may not be optimal<sup>47</sup>.

Finally, although the present study was performed using a crossover study design in which subjects were their own controls, the relatively small group of subjects may have resulted in a limited statistical power, especially with regard to the [<sup>123</sup>I]β-CIT binding in SERT-low cortical areas with its limited signal-to-noise ratios. Moreover, due to the low specific to nonspecific binding ratios in the cortical areas, one may expect that the test reliability is lower in these areas than in areas with high SERT densities. However, because of the radiation exposure we could not perform test-retest measurements to determine test reliability. The accuracy of this study would probably have been improved by coregistration of the SPECT images with MRI. Also, we did not check levels of citalopram in blood samples, although we supervised the intake of the citalopram or placebo capsule.

In conclusion, our results provide evidence that [<sup>123</sup>I]β-CIT SPECT is a valid technique to study SERTs *in vivo* in humans in SERT-rich brain areas, as binding ratios significantly decreased by pretreatment with the selective SSRI citalopram. In addition, we show that [<sup>123</sup>I]β-CIT also seems to bind to SERTs in SERT-low brain regions, such as the temporal and frontal cortex. Due to the very low specific binding in these areas, statistical significant changes in specific binding ratios due to citalopram were only detectable with a voxel-by-voxel analysis, and, therefore, *in vivo* measurements with [<sup>123</sup>I]β-CIT SPECT in cortical areas must be interpreted with caution. In addition, pretreatment with citalopram caused increased binding ratios in the DAT-rich striatum and increased absolute uptake in the cerebellum. More studies, especially in humans, are needed to evaluate the accuracy of the use of cerebellar binding to estimate nonspecific binding. Finally, in near future this study needs to be repeated with SPECT radiotracers that are more selective for SERTs than [<sup>123</sup>I]β-CIT, to investigate whether the selective radiotracers are more sensitive to assess SERTs in, especially, SERT-low brain areas.

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CHAPTER

6

# **Brain Kinetics of the New Selective Serotonin Transporter Tracer [<sup>123</sup>I]ADAM in Healthy Young Adults**

Nuclear Medicine and Biology 2006; 33: 185-191

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# ABSTRACT

## Background

Recently, the tracer  $^{123}\text{I}$ -2-([2-(dimethylamino)methyl]phenyl)thio-5-iodophenylamine ( $^{123}\text{I}$ ADAM) has been developed for selective imaging of serotonin transporters (SERTs) with single photon emission computed tomography (SPECT).

## Aim

To develop an  $^{123}\text{I}$ ADAM SPECT protocol for clinical studies in young adults.

## Material and Methods

We examined the time course of  $^{123}\text{I}$ ADAM binding to central SERTs in eight healthy young volunteers up to 6 h post-injection (p.i.).

## Results

We found that the time of peak-specific  $^{123}\text{I}$ ADAM binding was highly variable among subjects, but specific binding in the SERT-rich (hypo)thalamus peaked within 5 h p.i. in all subjects. Moreover, in this brain area, binding ratios of specific to nonspecific binding did not significantly change between 3 and 6 h p.i., and peaked 5 h p.i..

## Conclusions

The timepoint 5 h p.i. may be optimal for single-scan  $^{123}\text{I}$ ADAM SPECT studies in humans, but more work is needed to assess the accuracy of the 5 h tissue ratio as a measure of SERT in the brain.

## INTRODUCTION

Serotonin transporters (SERTs) are located in the membrane of serotonergic neuron terminals and play an important role in the regulation of the serotonin content. They are believed to be the primary target for antidepressants, such as selective serotonin reuptake inhibitors. A recent study has even shown that the central SERT availability predicts treatment response to this type of medication<sup>1</sup>. Disturbances in the central serotonin system are also thought to play an important role in psychiatric disorders, such as major depression or anxiety<sup>2-5</sup>. In addition, several neuropsychiatric diseases are characterized by loss of serotonergic function. For example, loss of SERT has been shown in Parkinson's disease, and the reductions in SERT binding correlated with ratings of disease severity<sup>6</sup>. Finally, the results of positron emission tomography (PET) and single photon emission computed tomography (SPECT) studies suggested that MDMA (ecstasy) may be neurotoxic for serotonergic neurons<sup>7,8</sup>. Therefore, *in vivo* imaging of SERTs with PET or SPECT provides an important tool to study the availability of SERTs in living human brain.

Until now, several selective radiotracers for the SERT have been developed and evaluated successfully for PET<sup>9-11</sup>. However, for clinical SPECT studies, the use of a selective SERT radiotracer has not been evaluated extensively yet. Therefore, until now, nearly all clinical SERT SPECT studies had to rely on the use of validated nonselective radiotracers, especially [<sup>123</sup>I]β-CIT or [<sup>123</sup>I]nor-β-CIT. These tracers are derived from cocaine, and not only label SERTs, but also dopamine transporters (DATs) *in vivo*<sup>12-14</sup>. A selective tracer for the SERT would have clear advantages over nonselective tracers, however, because also SERT-rich brain areas such as the midbrain contain not only SERTs but also DATs<sup>15</sup>.

Recently, the diarylsulfide derivative [<sup>123</sup>I]-2-((2-((di(methylamino)methyl)phenyl)thio)-5-iodophenyl)amine ([<sup>123</sup>I]ADAM) has been developed. *In vitro* studies have shown that this tracer has a high affinity for SERTs, whereas the affinity for DATs is low<sup>16</sup>. Moreover, *in vivo* biodistribution and autoradiographic studies in small laboratory animals have shown that the *in vivo* binding of this radiotracer is selective for SERTs<sup>17-19</sup>. In addition, preliminary studies in nonhuman primates and in human brain have shown that radiotracer binding in the midbrain is a specific binding to SERTs<sup>20,21</sup>. Based on these promising results, initial biodistribution studies in humans have been performed and showed that [<sup>123</sup>I]ADAM passes the blood-brain barrier and has an acceptable effective dose equivalent (0.022–0.037 mSv/MBq) to perform studies in humans<sup>21,22</sup>. Moreover, the results of recent studies suggested that an optimal imaging time for human [<sup>123</sup>I]ADAM SPECT studies may be around 4 to 6 h post-injection (p.i.)<sup>23,24</sup>. Because only data are available from a limited number of studies, the current study investigated the same topic. Therefore, we evaluated the optimal time p.i. of [<sup>123</sup>I]ADAM to perform clinical brain SPECT

studies in healthy volunteers. We focused on young adults because our research group is especially interested in studying the possible neurotoxic effects of ecstasy on the central serotonin system <sup>8</sup> and ecstasy is primarily used by subjects younger than 35 years.

## MATERIALS AND METHODS

### Subjects

To examine the brain kinetics of the tracer [<sup>123</sup>I]ADAM, eight healthy volunteers (four females and four males) were included in the present study. Exclusion criteria were age below 18 or above 35 years, major mental or physical problems, use of psychopharmaceuticals such as selective serotonin reuptake inhibitors, use of hard drugs such as ecstasy, cocaine, amphetamine or heroine in the past and pregnancy.

To exclude a clinical depression, all volunteers had to complete the Beck Depression Inventory (BDI), a validated self-report depression questionnaire, before each imaging session. Written informed consent was obtained from all subjects, and the study was approved by the local medical ethics committee.

### [<sup>123</sup>I]ADAM SPECT experiments

Subjects were examined using SPECT with the ligand [<sup>123</sup>I]ADAM <sup>16</sup>. Radiosynthesis of [<sup>123</sup>I]ADAM was performed at MAP Medical Technologies Oy (Tikkakoski, Finland). The <sup>123</sup>I-labeled ADAM was prepared by iododestannylation of the corresponding trimethyltin precursor, with carrier-free [<sup>123</sup>I] as NaI <sup>22</sup>. The radiochemical purity of the solution was always higher than 95.0% (as assessed with high-pressure liquid chromatography by the manufacturer). Approximately 185 MBq [<sup>123</sup>I]ADAM was injected intravenously as a bolus. Subjects received a potassium iodide solution to block thyroid uptake of free radioactive iodide.

### SPECT camera

Single photon emission computed tomography studies were performed using a 12-detector single slice brain-dedicated scanner (Neurofocus 810, which is an upgrade of the Strichmann Medical Equipment) with a full-width at half-maximum resolution of approximately 6.5 mm throughout the 20-cm field of view (<http://www.neurophysics.com>). In this study, the energy window was set at 135 to 190 keV. Attenuation correction of all images was performed as earlier described <sup>25</sup>. Images were reconstructed in 3D mode (<http://www.neurophysics.com>).

## Time-course study

A time-course study was performed to determine the optimal time p.i. for *in vivo* SERT imaging with [<sup>123</sup>I]ADAM SPECT. Each subject was imaged during six sessions. Session 1 started 10 min p.i., after positioning of the head of the participant in the camera with the canthomeatal line parallel to the beams from the gantry-mounted lasers, by multi-SPECT acquisitions (120 s/slice), from the canthomeatal line up to the vertex (interslice distance, 5 mm). In this session, approximately 14 to 19 slices were acquired in a 64 x 64 matrix. After the first session, the images were examined visually to determine which cluster of consecutive slices included slices with cerebellar activity as well as slices with (hypo)thalamic activity. The lower level of the cluster was defined by three consecutive transversal slices containing clear cerebellar activity, and the upper level of the cluster was identified by the most rostral slice containing (hypo)thalamic activity. This cluster of consecutive slices, containing approximately 12 to 14 slices, was scanned (acquisition, 120 s/slice; 5-mm interslice distance) in the following five sessions: Session 2 (starting 1.5 h p.i.), Session 3 (3 h p.i.), Session 4 (4 h p.i.), Session 5 (5 h p.i.) and Session 6 (6 h p.i.). In these sessions, efforts were made to position the subject's head in the head holder of the camera conform to the position at Session 1. The distances from the meatuses of the ears and from the orbital angles to the position of the laser beams were recorded.

## Image analysis

For quantification, a region-of-interest (ROI) analysis was performed. For this analysis, standardized templates of 2D ROIs were used, as shown and described extensively earlier<sup>26</sup>. This template included fixed ROIs for midbrain, (hypo)thalamus, striatum, frontal cortex, temporal cortex, occipital cortex, and cerebellum. The ROIs were positioned manually on the SPECT slices. Small variations of individual brains required movement of the fixed ROIs, without changing size and shape, within the template for optimal fitting. The ROIs for the frontal cortex and for the right and left occipital cortices were positioned on four consecutive slices: the three most rostral slices with the highest striatal activity and one slice above. We defined the caudal level of the (hypo)thalamus to begin at the first slice with the most caudal level of the striatum. For the (hypo)thalamic area, a template with an oval-shaped ROI was placed on four SPECT slices with the highest (hypo)thalamic activity. The upper level of the midbrain was defined to be at the first rostral slice without striatal activity visible. For this midbrain area, an ROI was placed on four SPECT slices with the highest activity of the midbrain. For the cerebellum, the ROI was placed on three SPECT slices with the highest activity of the cerebellum. Region of interests for the

right and left temporal cortex were placed on the two most rostral slices where the cerebellum template was positioned.

Mean striatal, mean temporal and mean occipital binding densities were calculated from the right and left ROIs. Absolute uptake in the ROIs was defined as the mean activity within the ROI (expressed as Bq per ml). To optimize comparability between subjects, we corrected these activity measures for individual body weight and injected dose (kg per MBq). Activity in the cerebellum was assumed to represent nondisplaceable activity (nonspecific binding and free radioactivity).

After peak-specific uptake in the region with the highest uptake was reached, the washout of activity per hour was calculated as [(specific activity 6 h p.i. - specific activity peak) / specific activity peak] divided by the number of hours between peak and end of the experiment (always 6 h p.i.), expressed as percentage. Washout of cerebellar activity per hour was calculated as [(cerebellar activity 6 h p.i. - cerebellar activity peak) / cerebellar activity peak] divided by the number of hours between peak and end of the experiment (which was always 6 h p.i.), expressed as percentage. Specific to nonspecific binding ratios were calculated as (activity in ROI - activity in cerebellum)/activity in cerebellum<sup>27</sup>.

## Statistical analysis

A repeated measures nonparametric test (Wilcoxon signed ranks test) was used to detect differences in specific to nonspecific ratios of [<sup>123</sup>I]ADAM binding in the brain area with the highest SERT binding between the different sessions. In the statistical analysis, which was two-tailed,  $p < 0.05$  was considered statistical significant.

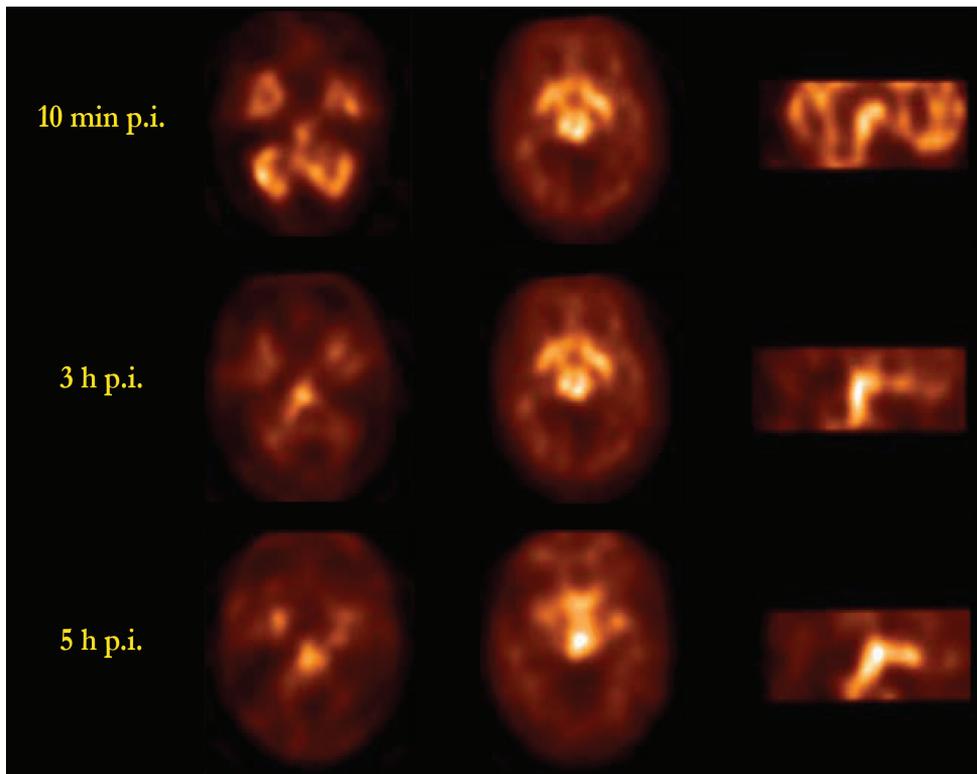
# RESULTS

## Participants

Mean age of the subjects was  $22.5 \pm 4.1$  years (range 18–30 years). Mean BDI scores were  $1.5 \pm 2.5$  (range 0–6). As BDI scores between 0 and 9 are regarded as normal, none of the subjects had a diagnosis of clinical depression.

## Time-course study

The SPECT images showed intense uptake of [<sup>123</sup>I]ADAM in human brain (Figure 1), especially at the level of the diencephalon and brainstem, during all SPECT sessions. Moreover, clear uptake was visible in the striatal area, especially at the early sessions, and to a lesser extent, in the (medial) temporal cortex. In other cortical

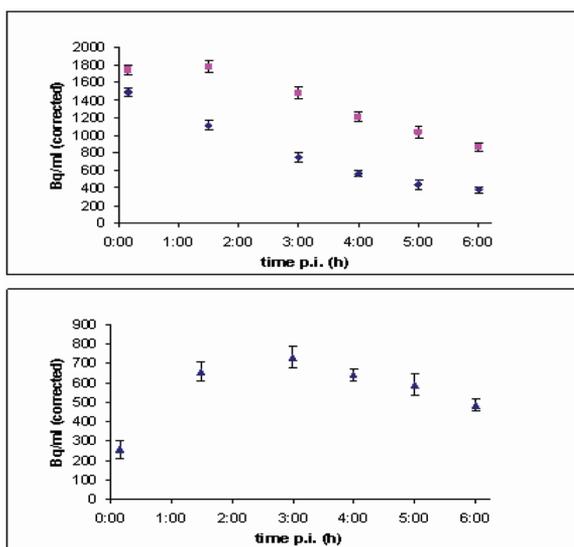


**Figure 1:** SPECT images of a male healthy volunteer, obtained 10 min, 3 h and 5 h p.i. of approximately 185 MBq [<sup>123</sup>I]ADAM. The left column represents transversal slices at the level of the cerebellum and temporal cortex. The middle column represents transversal slices at the level of the thalamus, striatum, frontal and occipital cortex. The right column represents sagittal slices approximately at the midline. The images are color encoded for low (black) to high activity (white). (See page 311 for colour illustration)

areas, uptake of the radiotracer was not substantially higher than in the cerebellum during all sessions.

The time-course curves obtained in the eight healthy controls showed most accumulation of specific activity in the (hypo)thalamus area. Specific activity in this area was very intense and peaked rapidly. Peak-specific binding was reached 3 h p.i. on average (Figure 2), but showed a large variation between subjects. Peak-specific binding was reached in all subjects 5 h p.i. (range 1.5–5 h p.i.). After peak-specific binding, the washout of specific activity was on average  $13 \pm 2\%/h$  (mean  $\pm$  S.E.M.). Also, in the midbrain area, intense activity was observed in all subjects. In this brain area, peak-specific binding was also reached on average 3 to 4 h p.i., and in all participants, the peak was reached 5 h p.i. of [<sup>123</sup>I]ADAM (data not shown).

In the temporal cortex and in the striatum, activity was higher than in the cerebellum, and also, in these areas, peak-specific binding was reached in all subjects



**Figure 2:** Upper panel, total radioactivity (expressed as becquerel per milliliter, corrected for injected dose and body weight (kg/MBq)) measured in the (hypo)thalamic area (■) and cerebellum (◆) (mean and S.E.M. of eight volunteers). Lower panel, specific radioactivity (which represents total activity minus activity measured in the cerebellum, corrected for injected dose and body weight) measured in the (hypo)thalamic area (mean and S.E.M. of eight volunteers). Note that scaling of the y-axis differs from the upper panel of the figure.

5 h p.i. (data not shown). Because the uptake in the occipital and frontal cortex was not substantially higher than in the cerebellum (Figure 1), specific binding could not be assessed adequately in these regions.

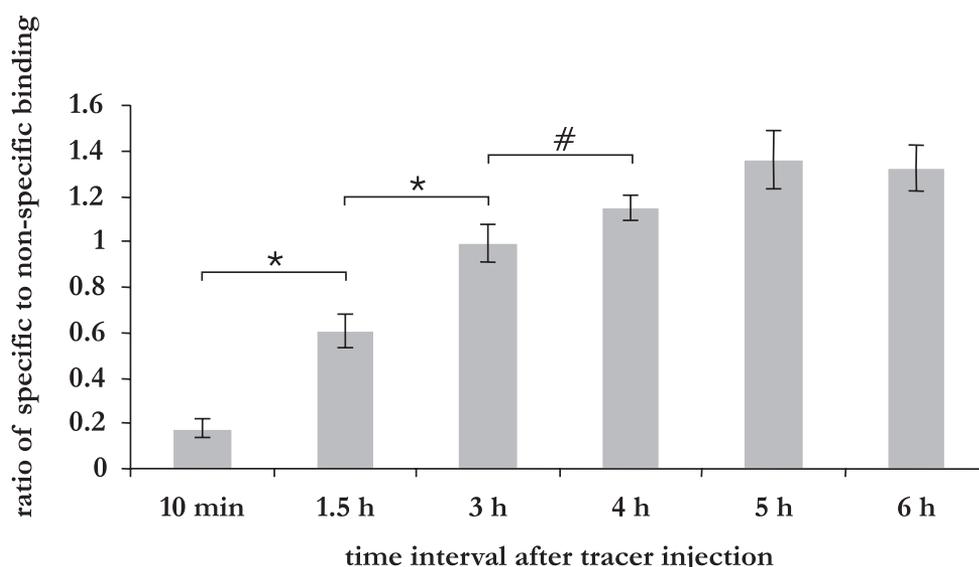
In the cerebellum, peak uptake was reached in all subjects 10 min p.i., which was followed by a fast, exponential, washout of activity ( $15 \pm 1\%/h$ , Figure 2).

## SPECT measurement

The mean ratio of specific to nonspecific [ $^{123}\text{I}$ ]ADAM binding was highest in the hypo(thalamus) area, and it reached its maximum at 5 h p.i. (Table 1). At that time, the ratio of specific to nonspecific binding was approximately 1.4 (Figure 3; Table 1). The ratio of specific to nonspecific binding was statistically significantly higher between 10 min and 1.5 h p.i., and between 1.5 and 3 h p.i. (Figure 3). From that moment on,

**Table 1:** Ratios of specific to nonspecific binding in various brain areas (mean  $\pm$  S.E.M.) up to 6 h postinjection of [ $^{123}\text{I}$ ]ADAM, in eight healthy young volunteers

	10 min	1.5 h	3 h	4 h	5 h	6 h
(Hypo)thalamus	$0.18 \pm 0.04$	$0.61 \pm 0.07$	$0.99 \pm 0.08$	$1.15 \pm 0.06$	$1.36 \pm 0.13$	$1.32 \pm 0.10$
Midbrain	$-0.12 \pm 0.05$	$0.30 \pm 0.06$	$0.58 \pm 0.09$	$0.78 \pm 0.10$	$0.87 \pm 0.13$	$0.83 \pm 0.11$
Striatum	$0.14 \pm 0.02$	$0.43 \pm 0.07$	$0.65 \pm 0.08$	$0.73 \pm 0.08$	$0.80 \pm 0.12$	$0.73 \pm 0.09$
Temporal cortex	$-0.22 \pm 0.07$	$0.16 \pm 0.06$	$0.31 \pm 0.04$	$0.38 \pm 0.05$	$0.47 \pm 0.07$	$0.43 \pm 0.06$
Frontal cortex	$-0.06 \pm 0.02$	$-0.01 \pm 0.05$	$0.06 \pm 0.03$	$0.17 \pm 0.03$	$0.27 \pm 0.05$	$0.29 \pm 0.07$
Occipital cortex	$-0.03 \pm 0.01$	$-0.07 \pm 0.05$	$-0.04 \pm 0.03$	$0.00 \pm 0.03$	$0.09 \pm 0.04$	$0.00 \pm 0.02$



**Figure 3:** Ratios of specific to nonspecific binding in the (hypo)thalamic area up to 6 h p.i. of [<sup>123</sup>I]ADAM (mean ± S.E.M.). \* Significantly different between data obtained 1.5 h p.i. vs. data obtained 10 min p.i., and of data obtained 3 vs. 1.5 h p.i.; # trend for statistical significance between data obtained 4 vs. 3 h p.i. of the radiotracer ( $p = 0.09$ ).

these ratios did not differ significantly, although a clear trend for significance was observed between 3 and 4 h p.i. ( $p = 0.09$ ).

At 5 h p.i., the ratio of specific to nonspecific binding in the hypo(thalamus) was not significantly different between females and males (Mann–Whitney U test); therefore, we calculated ratios only for the group as a whole. The mean ratio of specific to nonspecific binding in the midbrain area also increased up to 5 h p.i., and this ratio at peak was approximately 0.9 (Table 1). In the striatum and temporal cortex, the mean ratios were also at a maximum 5 h p.i., being 0.8 and 0.5, respectively (Table 1). In the frontal and occipital cortex, the specific uptake was not substantially higher than the nonspecific binding, resulting in ratios close to zero (Table 1).

## DISCUSSION

ADAM has recently been developed and showed high affinity selectively for the SERT<sup>16</sup>; it passes the blood–brain barrier and labels *in vivo* selectively for the SERTs<sup>9,10,17–19,28</sup>. In addition, initial human studies have shown that the dosimetry of [<sup>123</sup>I]ADAM is favorable<sup>21,22</sup>, and that an optimal imaging time for human studies may be around 4 to 6 h p.i.<sup>23</sup>. The current study also investigated the last topic to get additional evidence on the optimal time for single-scan ADAM SPECT studies in humans and to develop a SPECT protocol for routine clinical studies. This is important because

for patient's comfort, it is desirable that clinical SPECT studies are performed at one time point p.i. of a radiotracer during a short single session. Moreover, for optimal count statistics, it is, for an  $^{123}\text{I}$ -labeled tracer, preferable to start image acquisition within several hours p.i. Finally, a simple quantification procedure is recommended.

We found that peak-specific [ $^{123}\text{I}$ ]ADAM binding was reached in SERT-rich brain areas within 5 h p.i. in all subjects, and that this time point was highly variable among subjects. In addition, the ratios of specific to nonspecific binding were not significantly different from 3 to 6 h p.i. and reached a maximum 5 h p.i. Our results are largely confirmatory of a recent study published by Catafau *et al.*<sup>23</sup>, which found very similar results in a slightly larger series of healthy controls.

The "peak equilibrium method" is an adequate method to quantify receptors with SPECT or PET<sup>29</sup>. This method identifies the point in time of peak-specific binding to receptors/transporters. At that particular time point, the association and dissociation to and from the receptor/ transporter are equal. Consequently, at that point in time, the specific to nonspecific ratio derives an adequate measure of receptor/transporter density<sup>27</sup>. The results of the current study, however, showed that the time point of peak-specific (hypo)thalamic [ $^{123}\text{I}$ ]ADAM binding is highly variable among subjects. Thus, the peak equilibrium method is not practical for clinical [ $^{123}\text{I}$ ]ADAM SPECT studies, because an extended scanning period would be needed (1.5–5 h p.i.) to identify the time point of peak-specific [ $^{123}\text{I}$ ]ADAM binding per subject. From our present data, it becomes also clear that no true equilibrium for binding to SERTs was reached, as the washout of total and specific ADAM binding in the diencephalon as well as the cerebellar washout rates was relatively large. A secular equilibrium is reached at a time when the ratio of specific to nonspecific binding is stable<sup>30</sup>, and it provides an equivalent related to the density of available transporters. Fortunately, ratios derived during secular equilibrium are known to be linearly related to ratios obtained at true equilibrium<sup>30</sup>, and may provide an acceptable index of the density of transporters. Because we presently show that peak-specific hypothalamic binding was reached in all subjects 5 h p.i., that the ratios were stable between 3 and 6 p.i., and that the highest ratios were reached at 5 h, we recommend obtaining images 5 h p.i. in a clinical study with young adults.

In line with the previous imaging studies, using selective tracers for the SERT<sup>31,32</sup>, and with the necropsy data<sup>33</sup>, we found the highest specific [ $^{123}\text{I}$ ]ADAM binding in the hypo(thalamus) area and midbrain. In addition, specific striatal binding was substantially higher than nonspecific binding, also in agreement with the previous studies<sup>2,6,23,33</sup>. This finding may indicate the feasibility to image SERTs not only in brain areas dominated by SERTs, but also in brain areas with a higher density of DATs than SERTs, such as the striatum. Until now, such an approach was not feasible with nonselective SPECT tracers<sup>12,13,26</sup>. In the current study, the specific [ $^{123}\text{I}$ ]ADAM binding was substantially higher in the temporal cortex than in the

cerebellum. Visual inspection revealed that within this region, the highest binding was at the medial parts. This finding is in agreement with the results of recent human [ $^{123}$ I]ADAM SPECT studies<sup>2,23</sup>. It is tempting to speculate that the presently observed specific binding in the temporal cortex may represent binding to SERTs in limbic regions. Imaging of SERT in limbic areas may be of relevance for studying memory disorders<sup>34</sup>. For example, ecstasy users are at risk to develop disturbances in memory function related to both decreased SERT densities<sup>8</sup> and hippocampal dysfunction<sup>35</sup>. Unfortunately, in this study, we could not provide evidence that it is possible to reliably image SERTs with [ $^{123}$ I]ADAM SPECT in the hippocampus because we did not perform coregistration with MR images. Finally, in line with PET studies<sup>32</sup>, specific ADAM binding in frontal and occipital cortex was found to be very low, which precluded a reliable SERT measurement in these brain areas.

In this study, the cerebellum was used to assess nonspecific binding. Indeed, a recent autopsy study showed that the level of SERT is very low in the human cerebellum<sup>33</sup>. Moreover, several previous studies have used the cerebellum as a reference region because the specific binding appeared to be very low in this area<sup>32</sup>. However, one has to keep in mind that the cerebellum is not devoid of SERTs. In line with this, a recent PET study in baboons has shown that both the selective SERT tracers decreased significantly in the cerebellum after MDMA-induced 5-HT neurotoxicity with MDMA<sup>36</sup>. Moreover, a recent [ $^{123}$ I]ADAM rat study showed that cerebellar binding could be blocked with an SSRI<sup>17</sup>. Therefore, it has been suggested not to use the cerebellum as a reference region for SERT imaging<sup>36</sup>. In this context, Kish *et al.* recently pointed out that when a disease condition is associated with loss of cerebellar SERT, the specific binding in this region will also be lowered, leading to an elevated binding ratio and, consequently, an underestimation of the binding potential in the target region<sup>33</sup>. However, as also addressed by Kish *et al.*, even a complete blockage of SERT may have a minimal effect on SERT binding quantification<sup>33</sup>. They therefore concluded that many of the emerging SERT radiotracers could use the cerebellum as a reference region, provided that the nonspecific binding is not negligible, which is the case for [ $^{123}$ I]ADAM.

The time point at which equilibrium occurs depends on several factors, including peripheral clearance, association and dissociation rate constants and the number of binding sites<sup>37,38</sup>. In this context, the influence of the number of binding sites on the time point at which equilibrium occurs has been clearly shown for [ $^{123}$ I] $\beta$ -CIT SPECT studies. In human brain, [ $^{123}$ I] $\beta$ -CIT equilibrium is reached much faster in SERT-rich brain areas (approximately 4 h p.i.) than in the DAT-rich striatum (20 h p.i.)<sup>13</sup>. On the basis of this theory, one may expect that peak-specific binding of [ $^{123}$ I]ADAM is faster in brain areas with a low number of SERT as compared to an area with a higher number of SERTs. Unfortunately, the data of the current study showed no such correlation. The reason for this may be that the differences in binding

ratios were not dramatically different between SERT-rich regions (e.g., ratio of 1.4 in the (hypo)thalamic area) versus areas with a relatively low density of SERTs (e.g., ratio of 0.5 in the temporal cortex). As clearly demonstrated by simulation studies <sup>37</sup>, such differences should be relatively high to predict clear differences of the point in time at which equilibrium will be reached. Indeed, [<sup>123</sup>I]β-CIT SPECT studies in healthy controls have shown large differences in specific to nonspecific ratios in SERT-rich areas versus DAT-rich areas, being, for example, 1.3 at 4 h p.i. in the hypo(thalamus) and 9.5 at 24 h p.i. in the striatum <sup>26</sup>.

Several limitations of the current study should be discussed. First, we did not perform coregistration with MRI, which might increase the accuracy to detect SERTs in smaller, but relevant, brain areas. Second, in this study, the metabolism of [<sup>123</sup>I]ADAM was not determined. This may be of value because a preliminary study showed that metabolism may be different between individuals <sup>24</sup>. Third, because it is not acceptable to image participants continuously up to 6 h p.i., participants were repositioned on the camera bed several times. Such an approach will, by definition, induce additional variation in the data. However, previously, we used a similar approach and showed that the reproducibility for measuring striatal DATs with [<sup>123</sup>I]FP-CIT SPECT was high <sup>39</sup>. Fourth, a relatively small sample, including both young females and males, was included. A previous study showed a little, but significant, effect of gender on SERT binding <sup>40</sup>. Moreover, because SERT density may decrease by aging <sup>2,41</sup>, the conclusions of the present study may be applicable only to young adults.

In conclusion, we show that peak-specific [<sup>123</sup>I]ADAM binding is reached in SERT-rich brain areas in all studied healthy young volunteers within 5 h p.i. of the radiotracer. In addition, our results confirm the findings of a recent study <sup>23</sup> and show that the ratios of specific to nonspecific binding in the hypo(thalamus) are not significantly different from 3 to 6 h p.i. and reach a maximum of 5 h p.i.. Therefore, 5 h p.i. may be optimal for single-scan [<sup>123</sup>I]ADAM SPECT studies in humans, but more work is needed to assess the accuracy of a 5-h tissue ratio as a measure of SERTs in the brain.

## ACKNOWLEDGEMENTS

This study was supported by a grant from the Dutch Brain Foundation (Hersenstichting Nederland; grantnr. 11F03[2]32), The Hague, The Netherlands.

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**PART**

**III**

**Retrospective studies in heavy  
ecstasy users**

CHAPTER

7

# **Converging Evidence for Specific Toxic Effects of Ecstasy on the Thalamus using MR and SPECT Imaging**

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# ABSTRACT

## Background

Neurotoxic effects of ecstasy have been reported, although it remains unclear whether effects can be attributed to ecstasy, other drugs or a combination of these.

## Aim

To assess specific/independent neurotoxic effects of ecstasy and contributions of amphetamine, cocaine, and cannabis as part of the Netherlands XTC Toxicity (NeXT) study.

## Material and Methods

Effects of ecstasy and other substances were assessed with  $^1\text{H}$ -MR spectroscopy, diffusion tensor imaging, perfusion weighted imaging and [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT (serotonin transporters) in a sample ( $N = 71$ ) with variation in drugs used, using multiple regression analyses.

## Results

Ecstasy showed specific effects in the thalamus with decreased [ $^{123}\text{I}$ ] $\beta$ -CIT binding, suggesting serotonergic axonal damage, decreased fractional anisotropy, suggesting axonal loss, and increased cerebral blood volume probably caused by serotonin depletion. Ecstasy had no effect on brain metabolites and apparent diffusion coefficients.

## Conclusions

Converging evidence was found for a specific toxic effect of ecstasy on serotonergic axons in the thalamus.

## INTRODUCTION

Previous studies have suggested (serotonergic) neurotoxicity of the recreational drug ecstasy (3,4-methylenedioxymethamphetamine, MDMA) <sup>1,2</sup>. However, these findings are still debated because few studies adequately controlled for polydrug use, especially because almost all ecstasy users are poly-drug users <sup>3</sup>. The current study, part of the Netherlands XTC Toxicity (NeXT) study <sup>4</sup>, was designed to adequately control for polydrug use by including a sample with variation in type and amount of drugs used. The relatively low correlations between the use of ecstasy and other substances allowed to use linear multiple regression analysis to differentiate between the effects of ecstasy and of other substances without problems of multicollinearity. A combination of both single photon emission computed tomography (SPECT) and advanced magnetic resonance imaging (MRI) techniques was used to simultaneously assess structural and functional aspects of potential ecstasy-induced neurotoxicity.

## MATERIAL AND METHODS

### Subjects

In total 71 subjects were included in the current study. A detailed description of the recruitment procedure can be found in a special design paper on the NeXT study <sup>4</sup>. Recruitment aimed to include a sample of subjects, with variations in the amount and type of drugs used to keep correlations between the different drugs as low as possible. The sample included 33 heavy ecstasy users and 38 non-ecstasy users, but both ecstasy users as well as non-ecstasy users showed considerable variation in type and amount of other drugs taken, i.e., some heavy ecstasy users reported minimal use of other drugs such as cannabis, amphetamine or cocaine, whereas others were moderate or frequent users of one or more other psychoactive drug. Similarly, some ecstasy-naïve subjects used no drugs at all, whereas other ecstasy-naïve subjects reported incidental or frequent use of amphetamine and/or cocaine and/or cannabis. Subjects were recruited using a combination of targeted site sampling, advertisement and snowball sampling. All subjects had to be between 18 and 35 years of age. Exclusion criteria were severe medical or neuropsychiatric disorders, use of psychotropic medications affecting the serotonin system, pregnancy, use of intravenous drugs, and contraindications for MRI. Subjects had to abstain from use of psychoactive substances for at least two weeks and from alcohol for at least one week before examinations. Pre-study abstinence was checked with urine drug screening (enzyme-multiplied immunoassay for amphetamines, MDMA, opioids, cocaine, benzodiazepines, cannabis, and alcohol).

Besides SPECT and MRI, subjects underwent functional MRI and cognitive testing; results of these measurements will be reported in separate publications. Subjects were paid for their participation (€150 for 2 days). The study was approved by the local medical ethics committee and written informed consent of each subject was obtained according to the Declaration of Helsinki.

## Assessment of ecstasy use and potential confounders

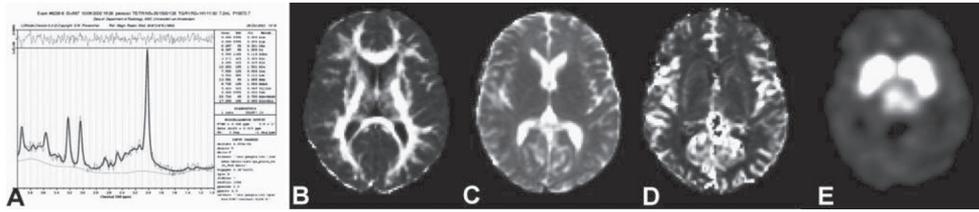
Lifetime use of ecstasy (number of tablets), cannabis (number of joints), amphetamines (number of occasions), cocaine (number of occasions), and use of alcohol (units/week) and tobacco (cigarettes/week) were assessed using substance-use questionnaires and the Substance Abuse Scales of the Mini International Neuropsychiatric Interview for DSM-IV clinical disorders (M.I.N.I.: Translated Dutch Version 5.0.0, <sup>5</sup>). Verbal intelligence was estimated using The Dutch Adult Reading Test (DART), i.e. the Dutch version of the National Adult Reading Test <sup>6</sup>.

## MRI acquisition and post-processing

*Acquisition:* MRIs were performed on a 1.5 T scanner (Signa Horizon, LX 9.0, General Electric Medical Systems, Milwaukee, WI) using the standard head coil. MRI acquisition, post-processing and quality control were performed with the same methods as used in another substudy of the NeXT study <sup>7</sup>. For completeness, we below summarize the most relevant aspects of the employed methods. The protocol included (1) axial PD- and T2-weighted imaging; (2) three voxel-based proton MR spectroscopy (<sup>1</sup>H-MRS) scans; (3) diffusion tensor imaging (DTI); (4) perfusion weighted imaging (PWI); and (5) high resolution T1-weighted 3D imaging. The <sup>1</sup>H-MRS voxels were placed in the left centrum semiovale (frontoparietal white matter) and in mid-frontal and mid-occipital grey matter in analogy to previous studies <sup>8,9</sup>. Throughout the study, positioning of subjects in the scanner and positioning of the slices and voxels were performed by the same examiner and according to a protocol in order to keep positioning as reproducible as possible.

*Post-processing:* Spectra derived from <sup>1</sup>H-MRS were analyzed using LCModel (Linear Combination of Model spectra) <sup>10</sup>. Ratios of N-acetylaspartate (NAA; neuronal marker), choline (Cho; reflecting cellular density) and myo-inositol (mI; marker for gliosis) relative to (phospho)creatine (Cr) were calculated.

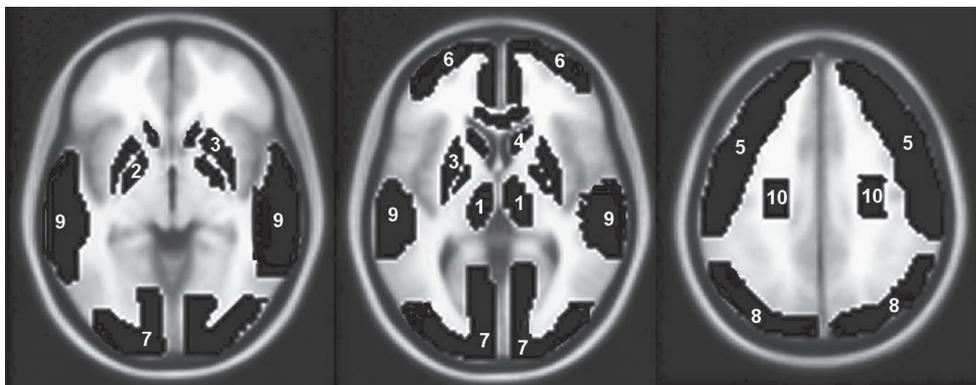
Apparent diffusion coefficient (ADC) and fractional anisotropy (FA) maps were calculated from the DTI <sup>11</sup> and cerebral blood volume (CBV) maps from the PWI scans. FA, ADC and CBV were spatially normalized by registration to the Montreal Neurological Institute brain template (MNI152), and segmentation was performed to separate into CSF, white and grey matter (Figure 1). The CBV maps were



**Figure 1:** Representative images of an individual (A)  $^1\text{H}$ -MRS after analysis by LCModel and representative (B) FA, (C) ADC, (D) rrCBV and (E)  $^{123}\text{I}$ β-CIT binding images after transformation to the spatially normalized MNI brain template.

intensity-scaled to mean individual CBV intensity of white matter derived from the segmentation procedure to generate relative CBV (rCBV) maps.

Regions of interest (ROIs) were drawn on the MNI152 brain template in thalamus, putamen, globus pallidus, head of the caudate nucleus, centrum semiovale (frontoparietal white matter), and dorsolateralfrontal, mid-frontal, occipital, superior parietal, and temporal cortex (Figure 2). Only grey matter voxels were included for the cortical ROIs, whereas white and grey matter voxels were included for in the ROIs of the basal ganglia (i.e. excluding CSF voxels). Selection of ROIs was based on findings of previous studies, which indicated that ecstasy-induced abnormalities are most prominent in basal ganglia and certain cortical areas; ecstasy-induced abnormalities in white matter were rarely reported and thus not expected. As cortical grey matter has very low anisotropy, it is very difficult to get reliable FA and ADC measurements in cortical areas. For this reason only ROIs in white matter and basal ganglia were taken into account in the measurements of FA and ADC. Within the ROIs, individual mean values of FA, ADC, and regional relative CBV (rrCBV) were



**Figure 2:** Region of interests used for analyses of DTI and PWI scans drawn on MR brain template at three levels: (1) thalamus, (2) globus pallidus, (3) putamen, (4) caudate nucleus, (5) dorsolateral frontal cortex, (6) mid-frontal cortex, (7) occipital cortex, (8) superior parietal cortex, (9) temporal cortex, and (10) white matter of the centrum semiovale. Note that rrCBV was measured in all these ROIs and FA and ADC only in the white matter and basal ganglia ROIs.

calculated. Values of FA, ADC and rrCBV from ROIs in left and right hemispheres were averaged.

## SPECT acquisition and post-processing

*Acquisition:* In a subgroup of the population ( $N = 47$ ) SPECT imaging was performed with the radioligand [ $^{123}\text{I}$ ] $\beta$ -CIT that binds to serotonin transporters (SERTs), dopamine transporters (DATs), and to a lesser extent also to norepinephrine transporters (NETs). The procedure of radiosynthesis of [ $^{123}\text{I}$ ] $\beta$ -CIT and acquiring of SPECT images were the same as previously described <sup>12</sup>. A bolus of approximately 110 MBq (3 mCi) [ $^{123}\text{I}$ ] $\beta$ -CIT was injected intravenously and SPECT images were acquired 4 h after the injection, when stable specific uptake to the SERTs is expected to be reached <sup>13</sup>.

*Post-processing:* Attenuation correction of all images was performed as previously described <sup>14</sup>. Images were reconstructed in 3D-mode (<http://www.neurophysics.com>). First, all SPECT scans were registered to the T1-3D MRI scans of the same person by maximizing mutual information and applying rigid transformations using self-developed software. Second, the individual MRIs were registered to the 152MNI brain using affine transformation and these transformations were applied to register the individual SPECT scans to the 152MNI brain template (Figure 1). This resulted in  $91 \times 109 \times 91$  voxel images, with voxel sizes of  $2 \times 2 \times 2 \text{ mm}^3$ . For quantification, both ROI and voxel-by-voxel analyses were performed. For the ROI analysis, regions were drawn on the 152MNI template in midbrain, thalamus, temporal cortex, frontal cortex, and occipital cortex. We did not measure SERT uptake in the putamen, caudate nucleus and globus pallidus, because there is no specific uptake to SERT or DAT in these regions 4 h after [ $^{123}\text{I}$ ] $\beta$ -CIT injection. Activity in the cerebellum was assumed to represent non-displaceable activity (non-specific binding and free radioactivity). Specific to non-specific binding ratios were calculated as (activity in ROI – activity in cerebellum)/ activity in cerebellum. The image registration was visually inspected to check the quality of this registration.

For the voxel-by-voxel analysis, the Statistical Parametric Mapping software (SPM2, Wellcome Department of Imaging Neuroscience, Functional Imaging Laboratory, London, UK; [www.fil.ion.ucl.ac.uk/spm](http://www.fil.ion.ucl.ac.uk/spm)) was used <sup>15</sup>. The registered scans were intensity-scaled to the corresponding mean cerebellar non-specific counts per voxel. The mean cerebellar counts were obtained from the ROI analysis. Then, smoothing was applied with SPM2 (Gaussian kernel with a 16 mm FWHM) to reduce interindividual anatomical differences that remained after stereotactical normalization <sup>16</sup>.

## Statistical analyses

### Substance use variables and potential confounders

Self-report histories of drug use may not be fully accurate and there is variation in the amount of MDMA in different ecstasy tablets. In addition, drug use variables in the current study were not normally distributed, not even after log-transformation. Therefore, all drug-use variables were dichotomized using a cut-off score to maximize contrast between users and non-users of a particular drug. For ecstasy, amphetamines and cocaine the cut-off score was arbitrarily determined at > 10 tablets/occasions lifetime. For cannabis, the cut-off score was set somewhat higher (> 50 joints lifetime), because experimenting with cannabis is much more common than with other illicit drugs. Table 1 shows cut-off values, frequency distributions, means ( $\pm$  SD) and medians for the substance variables in the total sample.

**Table 1:** Demographic features and drug usage patterns for the whole sample ( $N = 71$ )

	cut-off value for drug-use classification	$N = 71$	mean $\pm$ SD	median	range
Gender		44M/27F			
Age (years)		71	23.3 $\pm$ 3.8	22.6	18 - 37
IQ (DART-score)		71	101 $\pm$ 7.7	100	83 - 122
Ecstasy users	> 10 tablets lifetime	33/71	322 $\pm$ 354	250	15 - 2000
Amphetamine users	> 10 occasions lifetime	18/71	151 $\pm$ 154	120	15 - 600
Cocaine users	> 10 occasions lifetime	22/71	72 $\pm$ 70	43	12 - 300
Cannabis users	> 50 joints lifetime	42/71	1234 $\pm$ 1622	688	56 - 6650
Alcohol users	> 10 unit per week	36/71	22 $\pm$ 12	22	12 - 60
Tobacco users	> 10 cigarette per week	32/71	85 $\pm$ 46	80	17 - 200

Mean  $\pm$  SD, median and range for the different drugs show only values from subjects classified as user

Phi coefficients were calculated to assess the associations between dichotomized drug-use and demographic variables (Table 2). The relatively low association between some independent variables does not affect the validity of the regression model, because each regressor is adjusted for the predictive effect of all other regressors in the model. The variance inflation factor (VIF) was used to estimate multicollinearity. In the various analyses, VIF values ranged from 1.0 to 1.7, indicating that factor correlations did not cause over-specification of the regression model, allowing for reliable estimation of the independent effects of the various drugs on the neuroimaging parameters<sup>17</sup>.

Gender was included in all regression analyses because previous studies indicated that females are more vulnerable to the effects of ecstasy than males<sup>18,19</sup>. The most important potential drug-use confounders amphetamine, cocaine, and cannabis were included in all adjusted regression analyses. Additional confounders (age, verbal IQ,

**Table 2:** Phi correlations between dichotomized substance use variables in the whole sample ( $N = 71$ ).

	Age	Gender	DART-IQ	Alcohol	Tobacco	Ecstasy	Amph	Cocaine	Cannabis
Age		NS	NS	NS	NS	NS	NS	NS	NS
Gender			NS	NS	NS	NS	NS	NS	NS
DART-IQ				NS	NS	NS	NS	NS	<b>-0.23</b>
Alcohol					NS	NS	NS	NS	<b>0.38</b>
Tobacco						<b>0.40</b>	NS	<b>0.31</b>	<b>0.41</b>
Ecstasy							<b>0.43</b>	<b>0.54</b>	NS
Amphetamine								<b>0.45</b>	NS
Cocaine									NS
Cannabis									NS

Substance use variables are dichotomized (0 = below cut-off value; 1 = above cut-off value), see Table 1 for classification criteria.

NS = not significant

alcohol, tobacco) were chosen based on theoretical grounds per modality to reduce the number of regressors in the regression model: for  $^1\text{H-MRS}$ , verbal IQ was added as additional confounder because a relationship between brain metabolites and verbal IQ was reported <sup>20</sup>; for DTI no additional confounders were included in the analyses; and for PWI and SPECT imaging tobacco was added as an additional confounder, because previous studies showed a relationship between smoking and brain perfusion <sup>21</sup>, as well as between smoking and SERT densities <sup>22</sup>. Age was not included as a confounder, due to the relatively small age-range within the sample.

### Linear regression MRI and SPECT ROI analyses

To assess the specific effects of ecstasy and contributions of other drugs on the outcome parameters of MRI and SPECT imaging, linear multiple regression analyses were performed. Two different stepwise multiple linear regression models were used with imaging parameters as dependent variables.

Model 1 estimated the upper bound effect of ecstasy on outcome parameters, i.e. with adjustment for the effects of gender (and also verbal IQ in the case of  $^1\text{H-MRS}$ ), but without correction for the effects of other drugs. In the first step, gender (and IQ) was entered as independent variable and in a second step, ecstasy was entered. The effect of ecstasy was quantified as the  $R^2$ -change between the first and the second steps of the model. This model resembles the approach in previous studies that compared ecstasy users with non-users. However, the effect of ecstasy in this model is likely to be an overestimation of the real independent effect of ecstasy due to the lack of correction for the impact of other drugs on the imaging parameters of neurotoxicity.

Model 2 estimated the lower bound effect of ecstasy on outcome parameters after adjustment for the effects of gender, IQ, and the use of substances other than ecstasy.

In analogy to model 1, first gender (and IQ in case of  $^1\text{H-MRS}$ ) and substance use other than ecstasy (cannabis, amphetamines and cocaine in all analyses and tobacco in case of PWI and SPECT) were entered in the model as independent variables. In a second step, ecstasy was entered as additional independent variable. Similar to model 1, the independent effect of ecstasy use was quantified as the  $R^2$ -change between the first and second steps of the model. The effect of ecstasy in model 2 is presumably an underestimation of the real independent effect of ecstasy, due to possible over-correction for the effects of other drugs.

Linear regression analyses were performed using SPSS version 11.5, SPSS Inc., Chicago, IL, USA).  $P$ -values  $< 0.05$  were considered statistically significant. Besides  $R^2$ , unstandardized regression coefficients ( $B$ ) were used to reflect the predictive power of the different regressors. In Table 3  $B$ 's are reported with 95% confidence intervals (95% CI) and in the text with their two-tailed significance level ( $p$ -value).

### SPECT voxel-by-voxel analysis

For the voxel-by-voxel analyses we did not use the sample as a whole as in the other analyses, but divided the sample ( $N = 47$ ) into 5 groups, based upon the dichotomized drug-use variables (see Table 1). The groups included: 1) heavy ecstasy polydrug users ( $N = 10$ ); 2) selective ecstasy and cannabis users ( $N = 4$ ); 3) ecstasy-naive polydrug (amphetamine and/or cocaine and cannabis) users ( $N = 5$ ); 4) ecstasy-naive cannabis users ( $N = 16$ ); and 5) drug-naive controls ( $N = 12$ ). The [ $^{123}\text{I}$ ] $\beta$ -CIT binding ratios of the stereotactically- and intensity-normalized and smoothed SPECT images were compared between the 5 groups on a voxel-by-voxel basis by means of the spatial extent statistical theory using SPM2<sup>15,16</sup>. The PET/SPECT model 'single subject conditions and covariates' was chosen. Five conditions and no covariates were included. The main comparison was between ecstasy users and non-users (groups 1 and 2 versus groups 3, 4, and 5). Because this showed some significant clusters, post-hoc comparisons were made between the different groups to analyze whether significant differences were caused by ecstasy or by other substances. An effect was considered statistically significant if at least 20 connected voxels reached the one-sided  $p$ -value  $< 0.001$  ( $T = 3.30$ , uncorrected for multiple comparisons). Clusters of voxels surviving the thresholds were colour-coded and superimposed on the MNI152 template.

# RESULTS

## Sample characteristics and substance use

Characteristics of demography and substance use of the total sample are presented in Table 1. Mean cumulative dose of ecstasy within the ecstasy group was  $322 \pm 354$

**Table 3:** Linear regression imaging outcome parameters.

Imaging technique	Parameter	Region of interest	Model 1 <sup>a</sup>	
			Regression coeff <i>B</i> (95% CI) of ecstasy	R <sup>2</sup> ecstasy
<sup>1</sup> H-MRS (N=69)	NAA/Cr	mid-frontal grey matter	-0.017 (-0.137; 0.103)	0.2%
		mid-occipital grey matter	-0.013 (-0.113; 0.088)	0.1%
		left centrum semiovale	0.045 (-0.059; 0.148)	1.1%
	Cho/Cr	mid-frontal grey matter	-0.013 (-0.033; 0.007)	2.4%
		mid-occipital grey matter	0.008 (-0.007; 0.022)	1.5%
		left centrum semiovale	0.015 (-0.004; 0.034)	3.5%
	mI/Cr	mid-frontal grey matter	-0.053 (-0.144; 0.037)	2.0%
		mid-occipital grey matter	-0.041 (-0.100; 0.019)	2.8%
		left centrum semiovale	-0.018 (-0.095; 0.059)	0.3%
DTI (N=68)	FA	thalamus	<b>-20.09 (-30.91; -9.27)*</b>	<b>16.6%</b>
		globus pallidus	-10.48 (-31.73; 10.76)	1.4%
		putamen	-10.71 (-25.31; 3.90)	3.0%
		caudate nucleus	-14.32 (-29.96; 1.31)	4.7%
		centrum semiovale	-13.30 (-30.82; 4.21)	3.4%
		thalamus	2.18 (-1.08; 5.43)	2.4%
	ADC	globus pallidus	-0.78 (-1.97; 0.42)	2.5%
		putamen	-0.48 (-1.35; 0.39)	1.9%
		caudate nucleus	5.22 (-0.55; 10.98)	4.7%
		centrum semiovale	-0.98 (-2.18; 0.22)	4.0%
		thalamus	<b>0.094 (0.013; 0.176)*</b>	<b>7.3%</b>
		globus pallidus	-0.050 (-0.127; 0.027)	2.5%
PWI (N= 69)	rrCBV	putamen	-0.006 (-0.063; 0.052)	0.1%
		caudate nucleus	-0.006 (-0.067; 0.055)	0.1%
		dorsolateral frontal grey matter	0.056 (-0.002; 0.114)	5.3%
		mid-frontal grey matter	0.052 (-0.019; 0.122)	3.1%
		occipital grey matter	-0.077 (-0.180; 0.027)	2.9%
		superior parietal grey matter	-0.009 (-0.089; 0.071)	0.0%
		temporal grey matter	<b>0.111 (0.020; 0.202)*</b>	<b>8.1%</b>
		midbrain	-0.106 (-0.326; 0.113)	2.1%
		thalamus	<b>-0.394 (-0.570; -0.218)*</b>	<b>31.0%</b>
		frontal grey matter	<b>-0.090 (-0.152; -0.028)*</b>	<b>16.4%</b>
SPECT (N=47)	<sup>123</sup> I]β-CIT binding ratios	occipital grey matter	-0.029 (-0.101; 0.043)	1.5%
		temporal grey matter	<b>-0.160 (-0.254; -0.066)*</b>	<b>21.1%</b>

<sup>a</sup> linear regression analyses with ecstasy use (dichotomized) as independent variable

<sup>b</sup> linear regression analyses with ecstasy, amphetamines, cocaine and cannabis use (all dichotomized) as dependent variable, adjusted for covariates; \*  $p < 0.05$

tablets. Time since last ecstasy use within this group was  $8.2 \pm 9.8$  weeks, age at first use  $17.7 \pm 2.8$  years and usual ecstasy dose was  $2.7 \pm 1.6$  tablets per session.

## <sup>1</sup>H-MRS, DTI, and PWI

Two subjects had enlarged lateral ventricles, hampering matching to the MNI template, so the DTI and PWI of these subjects were not included. Due to technical

Model 2 <sup>b</sup>		
Regression coeff <i>B</i> (95% CI) of ecstasy	R <sup>2</sup> ecstasy	Significant predictors* other than ecstasy; regression coeff <i>B</i> (95%CI)
-0.056 (-0.200; 0.087)	0.9%	
-0.021 (-0.144; 0.103)	0.2%	
-0.004 (-0.127; 0.120)	0.0%	
-0.008 (-0.031; 0.016)	0.0%	cocaine -0.027 (-0.053; -0.002)
0.010 (-0.008; 0.028)	1.5%	gender -0.019 (-0.035; -0.003)
-0.000 (-0.023; 0.022)	0.0%	cocaine 0.027 (0.003; 0.051)
-0.004 (-0.112; 0.103)	0.0%	
-0.062 (-0.013; 0.008)	4.3%	amphetamine 0.085 (0.008; 0.162)
-0.088 (-0.176; 0.000)	5.4%	cocaine 0.144 (0.049; 0.239)
<b>-18.76 (-32.14; -5.39)*</b>	<b>9.7%</b>	gender -11.95 (-23.50; -0.40)
-14.06 (-40.58; 12.47)	1.7%	
-8.96 (-26.95; 9.03)	1.5%	
-15.27 (-34.36; 3.81)	3.6%	
-7.55 (-28.56; 13.46)	0.8%	amphetamine -24.53 (-47.02; -2.05)
2.68 (-1.36; 6.72)	2.4%	gender 4.84 (1.36; 8.33)
-0.75 (-2.20; 0.71)	1.6%	
-0.13 (-1.19; 0.94)	0.1%	
6.05 (-1.04; 13.15)	4.2%	
-1.10 (-2.59; 0.39)	3.3%	
<b>0.114 (0.007; 0.220)*</b>	<b>6.4%</b>	
-0.069 (-0.169; 0.031)	2.9%	
-0.010 (-0.083; 0.063)	0.1%	
-0.008 (-0.089; 0.072)	0.1%	
0.057 (-0.019; 0.133)	3.4%	
0.018 (-0.072; 0.108)	0.1%	
0.038 (-0.089; 0.165)	0.5%	gender -0.135 (-0.239; -0.031), tobacco -0.133 (-0.252; -0.014)
0.055 (-0.045; 0.156)	1.7%	amphetamine -0.109 (-0.212; -0.006)
<b>0.131 (0.013; 0.249)*</b>	<b>6.8%</b>	
-0.092 (-0.373; 0.188)	1.0%	
<b>-0.343 (-0.566; -0.121)*</b>	<b>15.2%</b>	
-0.058 (-0.135; 0.020)	4.3%	
0.021 (-0.060; 0.112)	0.8%	nicotine -0.078 (-0.155; -0.001)
-0.105 (-0.222; 0.012)	5.9%	

problems,  $^1\text{H-MRS}$  was not performed in two subjects and DTI in one subject. Therefore, we report measurements of  $^1\text{H-MRS}$  and rrCBV in 69 subjects and of FA and ADC in 68 subjects.

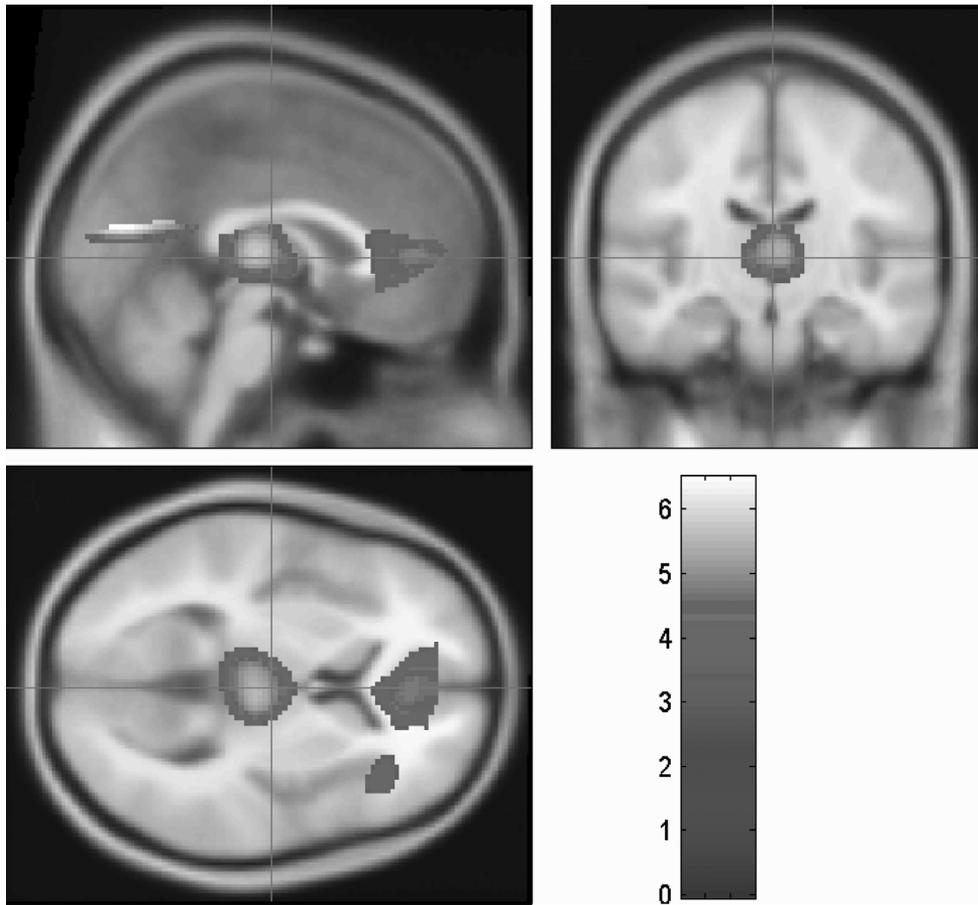
Table 3 shows results from the linear regression analyses. There was no significant effect of ecstasy use on the brain metabolites ratios NAA/Cr, Cho/Cr and mI/Cr in any of the three regions. With DTI no significant effects of ecstasy on ADC in basal ganglia were observed, but ecstasy did have a significant negative effect on FA in the thalamus (model 1:  $R^2_{\text{ecstasy}} = 16.6\%$ ;  $B_{\text{ecstasy}} = -20.09$ ,  $p < 0.001$ ). After adjusting for other drugs, the negative effect of ecstasy on thalamic FA remained significant (model 2:  $R^2_{\text{ecstasy}} = 9.7\%$ ;  $B_{\text{ecstasy}} = -18.76$ ,  $p = 0.006$ ). Also gender had a significant effect on FA in the thalamus (females had lower FA) ( $B_{\text{gender}} = -11.95$ ,  $p = 0.043$ ). Ecstasy had a significant positive effect on rrCBV in the thalamus (model 1:  $R^2_{\text{ecstasy}} = 7.3\%$ ;  $B_{\text{ecstasy}} = 0.094$ ,  $p = 0.024$ ) and the temporal cortex (model 1:  $R^2_{\text{ecstasy}} = 8.1\%$ ;  $B = 0.111$ ,  $p = 0.018$ ). These effects remained statistically significant after correction for other substances (model 2:  $R^2_{\text{ecstasy}} = 6.4\%$ ;  $B_{\text{ecstasy}} = 0.114$ ,  $p = 0.037$  for the thalamus and  $R^2_{\text{ecstasy}} = 6.8\%$ ;  $B_{\text{ecstasy}} = 0.131$ ,  $p = 0.030$  for the temporal cortex).

According to model 2, amphetamine had a positive effect on mid-occipital mI/Cr ratios ( $B_{\text{amphetamine}} = 0.085$ ,  $p = 0.031$ ), a negative effect on FA in the centrum semiovale ( $B_{\text{amphetamine}} = -24.53$ ,  $p = 0.033$ ) and a negative effect on rrCBV in the superior parietal cortex ( $B_{\text{amphetamine}} = -0.109$ ,  $p = 0.038$ ). Use of cocaine had a positive effect on both Cho/Cr ( $B_{\text{cocaine}} = 0.027$ ,  $p = 0.030$ ) and mI/Cr ( $B_{\text{cocaine}} = 0.144$ ,  $p = 0.004$ ) ratios in the centrum semiovale, whereas cocaine had a negative effect on Cho/Cr in the mid-frontal cortex ( $B_{\text{cocaine}} = -0.027$ ,  $p = 0.036$ ). Cannabis did not have any significant effect on MR outcome parameters.

## [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT

ROI analyses showed a significant negative effect of ecstasy on [ $^{123}\text{I}$ ] $\beta$ -CIT binding in the thalamus (model 1:  $R^2_{\text{ecstasy}} = 31.0\%$ ;  $B_{\text{ecstasy}} = -0.394$ ,  $p < 0.001$ ), frontal cortex (model 1:  $R^2_{\text{ecstasy}} = 16.4\%$ ;  $B_{\text{ecstasy}} = -0.090$ ,  $p = 0.005$ ), and temporal cortex (model 1:  $R^2_{\text{ecstasy}} = 21.1\%$ ;  $B_{\text{ecstasy}} = -0.160$ ,  $p = 0.001$ ) (Table 3). After adjustment for amphetamines, cocaine, cannabis, and tobacco (model 2), the effect remained significant in the thalamus ( $R^2_{\text{ecstasy}} = 15.2\%$ ;  $B_{\text{ecstasy}} = -0.343$ ,  $p = 0.003$ ), but not in the frontal and temporal cortex ( $p = 0.140$ , and  $p = 0.076$ , respectively). Amphetamine, cocaine and cannabis use did not have significant effects on [ $^{123}\text{I}$ ] $\beta$ -CIT binding in any of the ROIs.

Also with voxel-by-voxel analysis, lower [ $^{123}\text{I}$ ] $\beta$ -CIT binding was observed in the thalamus of ecstasy users compared to non-users ( $Z_{\text{max}} = 5.07$ ,  $p_{\text{corrected, cluster-level}} = 0.001$ ; coordinates highest Z-value: 2, -22, 8) (Figure 3). [ $^{123}\text{I}$ ] $\beta$ -CIT binding in the cingulate gyrus was also significantly lower in ecstasy users than in non-users,



**Figure 3:** Clusters of significantly lower  $[^{123}\text{I}]\beta\text{-CIT}$  binding ratios in ecstasy users compared to non-users, superimposed on the standard brain. The thalamic area showed the biggest cluster of significant difference ( $Z_{\text{max}}=5.07$ ,  $p_{\text{corrected, cluster-level}}=0.001$ ; coordinates highest Z-value: 2, -22, 8). A second significant cluster of decreased  $[^{123}\text{I}]\beta\text{-CIT}$  binding ratios in ecstasy users was observed in the cingulate gyrus, although this should be interpreted with caution, because the highest Z-value was exactly in the midline ( $Z_{\text{max}}=4.42$ ,  $p_{\text{corrected, cluster-level}}=0.007$ ; coordinates highest Z-value: 0, 42, 8). The clusters posterior on the sagittal slice are related to the upper boundary of the field of view and are likely to represent artefacts rather than real significant differences in  $[^{123}\text{I}]\beta\text{-CIT}$  binding. (see page 312 for colour illustration)

although this should be interpreted with caution, because the highest Z-value was exactly in the midline ( $Z_{\text{max}}=4.15$ ,  $p_{\text{corrected, cluster-level}}<0.001$ ; coordinates highest Z-value: 0, 42, 8). Post-hoc, the same cluster of significantly lower  $[^{123}\text{I}]\beta\text{-CIT}$  binding in the thalamus was observed in ecstasy users when we compared ecstasy users with substance-using controls (groups 1, 2 versus groups 3, 4), when we compared ecstasy polydrug users with ecstasy-naïve polydrug users (group 1 versus group 3), and when we compared selective ecstasy and cannabis users with ecstasy-naïve cannabis users (group 2 versus group 4). The cluster of significantly lower  $[^{123}\text{I}]\beta\text{-CIT}$  binding in

the anterior cingulate gyrus was observed in ecstasy users when we compared ecstasy users with substance-using controls (groups 1, 2 versus groups 3, 4) and when we compared selective ecstasy and cannabis users with ecstasy-naive cannabis users (group 2 versus group 4), but not when we compared ecstasy polydrug users with ecstasy-naive polydrug users (group 1 versus group 3). No clusters of increased [ $^{123}\text{I}$ ] $\beta$ -CIT binding were observed in ecstasy users in any of the comparisons. Selective ecstasy and cannabis users did not have clusters of significantly different [ $^{123}\text{I}$ ] $\beta$ -CIT binding than ecstasy polydrug users (group 1 versus group 2). Cannabis users did not significantly differ from drug-naives (group 4 versus group 5) and ecstasy-naive polydrug users did not differ on [ $^{123}\text{I}$ ] $\beta$ -CIT binding from drug-naive controls (group 3 versus group 5).

## DISCUSSION

Use of the party-drug ecstasy has been associated with decreased serotonergic function as shown by decreased densities of the SERT in membranes of serotonin axons, decreased neurocognitive performance, and increased depression scores in ecstasy users <sup>1,2,23</sup>. However, the validity of findings suggesting ecstasy-related neurotoxicity in humans is debated because most studies have methodological limitations <sup>24</sup>, including inadequate control of potential confounders such as polydrug use. The present study was designed to overcome limitations of previous studies, by adequately controlling for polydrug use and by combining, for the first time, advanced MR and SPECT imaging techniques in the same sample to study different aspects of brain involvement.

### Polydrug confounding in human ecstasy studies

Because almost all ecstasy users are polydrug users <sup>3</sup> it is difficult to differentiate effects of ecstasy from potential effects of other psychoactive drugs. Some studies reported that signs of neurotoxicity in ecstasy users might not be related to merely ecstasy use but rather to polydrug-use or the use of other psychoactive drugs, such as cannabis, amphetamines, or cocaine <sup>25</sup>. Only some of the previous studies adequately controlled for use of drugs other than ecstasy by including a group of 'pure' ecstasy users <sup>26,27</sup>, by including a drug-using but ecstasy-naive control group e.g. <sup>19,28</sup> or by statistically adjusting for polydrug use <sup>29,30</sup>. However, these attempts have limitations because 'pure' ecstasy users are very rare <sup>3</sup> and drug use in the control groups was generally lower than in the ecstasy groups and mainly comprised the use of cannabis and much less the use of amphetamines and cocaine. Controlling for polydrug use in a statistical regression analysis was generally hampered by the fact that cannabis,

cocaine, and amphetamine use were almost always strongly correlated with ecstasy use, leading to multicollinearity and the impossibility of statistical adjustment for these potential confounders in multiple regression analysis <sup>29</sup>.

In the current study we used a new approach by including a carefully selected sample of drug users with specific variations in amount and type of drugs used. This strategy successfully reduced the magnitude of the correlations between ecstasy use and the use of other substances and allowed to use linear multiple regression analysis to differentiate between the effects of ecstasy and of other substances.

## Specific effects of ecstasy to the thalamus

The most interesting finding is that different imaging techniques all showed a specific effect of ecstasy on the thalamus. Even after adjustment for amphetamine, cocaine, cannabis, and other relevant potential confounders, a significant effect of ecstasy, and no effects of any of the other drugs, was found on [<sup>123</sup>I]β-CIT binding (reduced), FA (reduced) and rrCBV (increased) in the thalamus. As [<sup>123</sup>I]β-CIT SPECT was previously validated to assess *in vivo* binding to SERT, the finding of decreased [<sup>123</sup>I]β-CIT binding probably reflect lower SERT densities in ecstasy users <sup>12,31,32</sup>. Moreover, the thalamus is a SERT-rich area and previous studies showed that [<sup>123</sup>I]β-CIT binding in the thalamus is mainly related to binding to SERTs, although the thalamus also contains NETs <sup>33</sup>. DTI measures diffusional motion of water molecules in the brain which is normally restricted in amplitude and direction by cellular structures such as axons <sup>34</sup>. When axons are damaged, extracellular water content increases and FA decreases. Therefore, it is likely that the observed decreased FA is related to ecstasy-induced axonal injury. An alternative explanation could be that decreased FA relates to increased brain perfusion in the thalamus, which also gives an increase in extracellular water content. As ecstasy was previously shown to reduce extracellular serotonin and serotonin is involved in regulation of brain microcirculation, mainly as a vasoconstrictor <sup>35</sup>, ecstasy-induced serotonin-depletion may have led to vasodilatation and the observed increase in rrCBV. Taken together, it seems that these measurements in the thalamus converge in the direction of decreased serotonergic function, with decreased SERT binding and decreased FA values probably reflecting damage to serotonergic axons and increased rrCBV due to decreased vasoconstriction caused by depletion of serotonin. Previous studies in animals also showed ecstasy-induced axonal damage to the serotonergic axons of the thalamus, although signs of reinnervation after a period of recovery were also observed <sup>36</sup>. As the thalamus plays a key role in awareness, attention, and neurocognitive processes, such as memory and language <sup>37</sup> one can speculate that ecstasy-induced serotonergic damage to the thalamus is (partly) responsible for reduced verbal memory performance frequently reported in ecstasy users .

## Integration with prior SPECT studies

Previous imaging studies in ecstasy users mainly used PET or SPECT techniques with tracers that bind to the SERT (for review see <sup>1</sup>). In line with the current study, almost all of these studies reported decreased SERT binding in the thalamus of ecstasy users <sup>18,19,38</sup>. However, most of these studies also reported lower SERT binding in other sub-cortical and cortical areas, although these areas varied in different studies. When only adjusted for gender and not for other substances, we also observed lower [<sup>123</sup>I]β-CIT binding in ecstasy users in the frontal cortex, mainly located in the anterior cingulate gyrus as shown by the voxel-by-voxel analysis, and temporal cortex. However, decreased [<sup>123</sup>I]β-CIT binding in these areas seemed to be related to polydrug use in general, and not to ecstasy or any other drug in particular, because none of the psychoactive substances was a significant predictor in the adjusted model. Moreover, decreased [<sup>123</sup>I]β-CIT binding ratios in SERT-low areas such as the cortical areas should be interpreted with caution <sup>12</sup>. We did not observe decreased [<sup>123</sup>I]β-CIT binding in midbrain and occipital cortex as previously observed and we could not reproduce findings that women might be more susceptible than men for the effects of ecstasy on the serotonergic system <sup>18,19</sup>.

## Integration with prior MR studies

Only few previous studies used advanced MR techniques to assess ecstasy-induced neurotoxicity. One preliminary study measured ADC in ecstasy users, although not in the thalamus, and reported an increased ADC in the globus pallidus of ecstasy users suggesting axonal damage <sup>39</sup>. In the current study we did not find any effect of ecstasy on ADC measurements. The same study of Reneman *et al.* <sup>39</sup> (not including measurements in the thalamus) also examined brain perfusion and showed increased rrCBV values in the globus pallidus of ecstasy users. Another study of the same group reported increased rrCBV values in the globus pallidus and thalamus of two former ecstasy users who were abstinent for 18 weeks on average <sup>40</sup>. In the current study we did not observe increased rrCBV values in the globus pallidus. However, we observed an increased rrCBV, only related to ecstasy and not to other drugs, in the thalamus and also in the temporal cortex, an area that was not included in the previous studies. Cerebrovascular changes in ecstasy users were also observed in a previous SPECT study, measuring regional cerebral blood flow (rCBF) <sup>8</sup>.

With <sup>1</sup>H-MRS, we did not find indications of neuronal damage (i.e., no decrease in NAA/Cr and no increase in Cho/Cr and mI/Cr in ecstasy users. However, we did not perform <sup>1</sup>H-MRS in the thalamus, because it is technically difficult to get reliable <sup>1</sup>H-MRS measurements in that area due to magnetic field inhomogeneities and because of partial volume effects. Previous studies showed lower NAA/Cr ratios

in the frontal cortex of ecstasy users with an average cumulated dose of more than 700 tablets, probably reflecting neuronal loss<sup>9,41</sup>, whereas others found no difference in NAA/Cr ratios in cortical brain regions in subjects with more moderate lifetime doses<sup>42,43</sup>. Therefore, these effects may only become apparent after very heavy ecstasy (polydrug) use. An increased mI in parietal white matter was only observed in one study (Chang *et al.*, 1999a).

## Effects of other drugs than ecstasy

In addition to the effects of ecstasy, the current study design enabled the exploration of the contributions of various other substances to the outcome parameters. Amphetamine use, which is mainly D-amphetamine in the Netherlands, also showed some significant effects on the outcome parameters. However, the different imaging techniques showed effects of amphetamine in different brain areas, and therefore these findings are less consistent than the converging findings of the ecstasy effects on the thalamus. Amphetamine users showed increased mI/Cr in the mid-occipital grey matter and decreased FA in the centrum semiovale, and decreased rrCBV in the superior parietal grey matter. As it is known that D-amphetamine use is mainly associated with dopaminergic toxicity<sup>44,45</sup>, these effects may be related to damage of the dopaminergic system. Cocaine had a positive effect on Cho/Cr and mI/Cr in the centrum semiovale, which might be related to increased glial activation. In contrast, cocaine had a negative effect on Cho/Cr in the mid-frontal grey matter. A previous study in cocaine users showed increased mI/Cr in both frontal grey and white matter, besides a decreased NAA/Cr in the frontal cortex<sup>46</sup>. Cocaine did not have any significant effect on outcomes of DTI, PWI, or SPECT measurements. Cannabis use had no significant effect on any of the outcome parameters. Also other studies showed little evidence that chronic cannabis use causes permanent brain damage<sup>47</sup> or changes in cerebral blood flow<sup>48</sup>, although there are indications that mild cognitive impairment can occur in very heavy chronic cannabis users<sup>49</sup>.

## Limitations of the study

Inherent to its cross-sectional design and lack of baseline data it is difficult to draw firm conclusions regarding the causality of the observed relationships between ecstasy use and the neuroimaging outcome parameters, because it is possible that differences between ecstasy users and controls were pre-existent<sup>50</sup>. We had to rely on the retrospective self-reported records of drug use in the past using drug history questionnaires. Moreover, there will have been variation in dosage and purity of ecstasy tablets, although pill-testing confirms that in the Netherlands 95% of the tablets sold as ecstasy contain MDMA as a major component<sup>51</sup>. Also environmental

circumstances under which ecstasy was taken and simultaneous use of other substances were heterogeneous. With this selection strategy we succeeded in creating relative independent factors for ecstasy and cannabis use, although the resulting correlations between use of ecstasy and amphetamine and cocaine were relatively low but still substantial and statistically significant. Nonetheless, correlations between use of ecstasy and other illicit drugs were lower than usually found after random recruitment among frequent ecstasy users (Scholey et al, 2004; Parrot et al, 2002) and statistical collinearity analyses did not suggest any problems of multicollinearity indicating that the regression model allowed for reliable estimation of the effects of the various drugs. Moreover, the association between ecstasy use and its most commonly used co-drug cannabis was successfully removed as a result of sample stratification, thereby controlling for an important confounder. Finally, we did not correct for multiple comparisons in order to minimize the risk of false negative results (type II errors)<sup>52</sup>. The use of various imaging techniques and assessments in different brain regions may have introduced some false positive findings (type I errors).

## Conclusions

This study, examining independent effects of ecstasy on the brain with different imaging techniques in the same sample shows a very specific and remarkable converging effect of ecstasy on FA, rrCBV, and [<sup>123</sup>I]β-CIT binding in the thalamus, indicative of decreased serotonergic function. This strongly suggests a specific toxic effect of ecstasy on serotonergic axons in the thalamus. Although it is not clear yet whether these effects are permanent, public health measures should be taken to prevent heavy recreational use of ecstasy.

## ACKNOWLEDGEMENTS

The NeXT study was supported by a grant of the Netherlands Organization for Health Research and Development as part of their Program Addiction (ZonMw 310-00-036). Silvia D. Olabarriaga participates in the Virtual Laboratory for e-Science project, which is supported by a BSIK grant from the Dutch Ministry of Education, Culture and Science (OC&W). We thank Prof. M. Moseley (Lucas MRS Center, Stanford University) for support in the implementation of the DTI protocol. The authors thank Dirk Korf for his help on the design of the study; Hylke Vervaeke for recruiting volunteers; Sarah Dijkink and Ivo Bisschops for assistance with data collection; Jacco Visser and other technicians for making the SPECT scans; Benoit Faivre, Dick Veltman, Matthan Caan, Frans Vos, Marcel van Herk and Jan Habraken for their help on analyzing the SPECT data; Erik-Jan Vlieger and Jeroen Snel for

their help on the post-processing of the DTI and PWI scans; Charles Majoie for reading all anatomical MR scans; and Maarten Koeter and Ben Schmand for their advice on the statistical analysis.

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# CHAPTER 8

# **Mood Disorders and Serotonin Transporter Density in Ecstasy Users - the Influence of Long-Term Abstinence, Dose, and Gender**

Psychopharmacology 2004; 173: 376-382

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# ABSTRACT

## Background

Neurotoxic effects of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') on the serotonin (5-HT) system were described in animals and humans, but little is known about long-term effect of ecstasy use on mood.

## Aim

To investigate short-term and long-term effects of ecstasy use on mood and its association with serotonergic neurotoxicity, dose, and gender in humans.

## Material and Methods

Fifteen moderate ecstasy users, 23 heavy ecstasy users, 16 former heavy ecstasy users and 15 drug-using, but ecstasy-naïve controls were included. Mood was assessed using the Composite International Diagnostic Interview (CIDI) and the Beck Depression Inventory (BDI). Outcomes were correlated with serotonergic transporter (SERT) density, assessed with [ $^{123}\text{I}$ ] $\beta$ -CIT single photon emission computed tomography (SPECT).

## Results

The prevalence of mood disorders assessed by CIDI did not differ between all groups. The overall test for differences in BDI scores between groups was near significance ( $p = 0.056$ ), with BDI scores higher in former heavy ecstasy users than in ecstasy-naïve controls ( $p = 0.045$ ). BDI scores were correlated with the total number of ecstasy tablets used ( $r = 0.310$ ;  $p = 0.021$ ). No associations between CIDI or BDI outcomes and SERT density or gender were observed.

## Conclusions

These results suggest that ecstasy use is not associated with clinical depression (CIDI). However, the number of ecstasy tablets taken lifetime was associated with higher BDI scores for depressive mood, and this relationship seemed to persist after ecstasy use had stopped. We did not find that depressed mood in ecstasy users was associated with decrease in SERT density. Prospective studies are needed to establish the causal relationship between ecstasy use and depressed mood.

## INTRODUCTION

Neurotoxic effects of 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) on the serotonin (5-HT) system have been extensively studied in animals (e.g. <sup>1</sup>). In addition, possible effects in humans were reported (e.g. <sup>2-5</sup>). As serotonin modulates many neuropsychological processes, including mood, we hypothesised that in ecstasy users serotonin deficits are associated with increased prevalence of mood disorders.

Previous research on the functional consequences of serotonergic neurotoxicity induced by ecstasy showed converging evidence of memory impairments <sup>6-8</sup>. However, studies on the effect of ecstasy use on mood are less conclusive. Several case reports described individuals who developed psychiatric problems associated with the use of ecstasy, including depression <sup>9,10</sup>. Other studies, however, reported no difference in prevalence of depression between ecstasy users and non-users <sup>11,12</sup>.

As a short-term effect, enhanced mood was described to occur shortly after ecstasy consumption <sup>13,14</sup>, followed by lowered mood a few days later <sup>15,16</sup>. Women were found to be more susceptible than men to this mid-week low mood <sup>17</sup>.

Studies in abstinent ecstasy users reported inconsistent results concerning the effect of ecstasy on mood. Krystal and co-workers reported no indication of clinical depression in ecstasy users after abstinence of psychoactive drugs for at least 3 weeks <sup>18</sup>. However, higher depression scores were found in one-week abstinent heavy ecstasy users than in controls <sup>19,20</sup>. Higher depression scores in moderate ecstasy users reported by Verkes and co-workers appeared not to be significant after correction for confounders <sup>21</sup>.

Only a few studies examined the long-term effect of ecstasy on mood, and their results seem to suggest that (former) heavy use of ecstasy is associated with increased symptoms of depression. MacInnes *et al.* examined the long-term effect of ecstasy on mood in former chronic, but currently moderate ecstasy users and found mild depressive symptoms in this group <sup>22</sup>. Gerra *et al.* found mood changes (dysphoria) 3 weeks <sup>23</sup> and 12 months <sup>24</sup> after ecstasy discontinuation. Morgan and co-workers <sup>25</sup> found elevated depression scores in ecstasy users and former ecstasy users who had stopped using ecstasy for at least 6 months. However, these elevated depression scores correlated only with the amount of cannabis they also used and not with the number of ecstasy tablets.

Because all studies were conducted retrospectively, it is not certain whether the observations of increased depressive symptoms in (former) ecstasy users represents a pre-existing condition, or is caused by serotonin deficits induced by ecstasy, or is the result of a combination of the two. Lieb and co-workers <sup>26</sup> studied the (temporal) relationship between ecstasy use and mental disorders in a representative sample of adolescents and young adults in a prospective follow-up study. They reported increased prevalence of mood disorders in ecstasy users compared to non-users, but found that the mood disorder preceded the first use of ecstasy in about 45% of

persons with major depression and in 70% of persons with dysthymia. However, information about the onset of ecstasy use and the onset of mood disorders prior to baseline was obtained retrospectively.

In approaching this problem of the relationship between ecstasy use and mood disorders, we thought it would be relevant to link depression scores in (former) ecstasy users with markers of serotonergic neurotoxicity. Serotonergic neurotoxicity can be assessed in the living human brain by measuring serotonin transporter (SERT) density. SERT is a structural element of the pre-synaptic membrane, and has been shown to be a reliable marker of MDMA-induced serotonergic neurotoxicity<sup>27</sup>. SERT density can be assessed using the radiotracer [<sup>123</sup>I]β-CIT that binds with high affinity to SERTs and dopamine transporters (DATs)<sup>28</sup> in combination with single-photon emission computed tomography (SPECT)<sup>29</sup>. Previous [<sup>123</sup>I]β-CIT SPECT studies showed loss of SERTs in subjects with a history of ecstasy use<sup>2</sup> and we found the same also in the population of the current study<sup>4,5</sup>.

To our knowledge, only one study has investigated both mood disorders and serotonergic neurotoxicity by measuring SERT densities using positron emission tomography (PET)<sup>30</sup>. In this study significantly elevated depression scores were associated with the number of exposures to ecstasy, in recent as well as in former ecstasy users (who had stopped using ecstasy for at least 5 months) when they were compared to the scores of drug-naïve controls. Evidence for serotonergic neurotoxicity was only observed in central serotonergic brain regions in current, but not in former ecstasy users. The authors did not investigate the association between serotonergic neurotoxicity (SERT availability) and depression scores.

The objective of the present study was to investigate short-term as well as long-term effects of ecstasy use on mood, and its association with SERT densities. Moreover, as there are indications that the effects of ecstasy are dose-dependent, and that female users are more susceptible than male users<sup>4,17,31</sup>, the influence of dose and gender were investigated as well.

## MATERIAL AND METHODS

### Participants

The present study population and sub-sets of it were described in previous publications in which the effects of ecstasy on SERT<sup>4</sup>, on DAT densities<sup>32</sup> and on memory function<sup>5</sup> were analysed. In brief, 69 subjects, aged 18 to 45 years, participated in the study and were divided into four subgroups: 15 moderate ecstasy users, 23 heavy ecstasy users, 16 former heavy ecstasy users and 15 controls that used drugs, but were ecstasy-naïve. Lifetime use of more than 50 tablets was defined as 'heavy' ecstasy

use, whereas lifetime use less than 50 tablets was defined as moderate ecstasy use. Individuals who had used at least 50 tablets lifetime but had stopped using ecstasy at least 12 months prior to the study were included in the subgroup of former heavy ecstasy users. Both ecstasy users and controls were recruited by means of flyers at locations in the Netherlands associated with the “rave scene” with the help of UNITY, a Dutch agency that provides harm-reduction information and advice about drugs.

Participants agreed to abstain from all psychoactive drugs for at least 3 weeks before the study. On the day of examination urine drug-analysis was performed with an enzyme-multiplied immunoassay for amphetamines, barbiturates, benzodiazepine metabolites, cocaine metabolite, opiates, and marijuana. Exclusion criteria were positive drug screening, pregnancy, medical or neuropsychiatric illness that impeded informed consent, and current use of anti-depressive medication that could compete with [ $^{123}\text{I}$ ] $\beta$ -CIT for SERT binding, such as selective serotonin re-uptake inhibitors. All subjects completed the Dutch version of the National Adult Reading Test (NART) that provides an estimate of premorbid IQ<sup>33</sup>. Written informed consent was obtained from all participants and the study was approved by the local Medical Ethics Committee.

## Outcome measures of mood

Lifetime and current diagnoses for mood disorders, including major depression, dysthymia and bipolar disorders, were assessed in all subjects using a computerised version of the Composite International Diagnostic Interview (CIDI, lifetime version 2.1)<sup>34</sup>. The CIDI is a fully structured interview that covers the criteria for diagnoses of the fourth edition of the Diagnostic and Statistical Manual of Mental Disorder (DSM-IV)<sup>35</sup>.

Additionally, current depression was assessed using the Beck Depression Inventory (BDI)<sup>36</sup>. The BDI is a 21-item self-report rating inventory, which measures characteristic attitudes and symptoms of depression in the week prior to assessment; higher scores indicate more depressive symptoms. The BDI showed high levels of reliability and validity across cultures<sup>37-39</sup>.

## SPECT imaging

As described previously,  $^{123}\text{I}$ iodine-2 $\beta$ -carbomethoxy-3 $\beta$ (4-iodophenyl)tropane ([ $^{123}\text{I}$ ] $\beta$ -CIT) SPECT was used to measure SERT densities<sup>4,5</sup>. SPECT imaging was performed with a dedicated 12-detector single-slice head scanner with a spatial resolution of 7.5 mm full-width at half-maximum (Strichman Medical Equipment 810, Inc, Medfield, Mass).

Subjects were intravenously injected with approximately 3.8 mCi (140 MBq) of [<sup>123</sup>I]β-CIT. Scanning was started 4 h after injection when the specific binding to SERT is regarded to be stable<sup>40</sup>. Subjects lay in supine position with the head parallel to the orbitomeatal line. Image analysis was performed using regions of interest (ROIs) for frontal cortex, temporal cortex, parieto-occipital cortex, occipital cortex, striatum, thalamus and cerebellum, as described earlier<sup>4,5</sup>. Binding ratios of [<sup>123</sup>I]β-CIT were calculated by dividing binding in the ROI by binding in the cerebellum.

## Statistics

We used a two-way analysis of variance with group and gender as factors to analyse differences in continuous variables (log transformed if necessary) between the four groups.

Differences in prevalence of lifetime and current mood disorders between the four groups, measured by CIDI, were analyzed using the Chi-Square test. Overall differences in BDI scores between the four groups were analyzed using the non-

**Table 1:** Demographics, characteristics of ecstasy use and exposure to other substances expressed as mean ± SD

	Polydrug controls (N=15)		Moderate ecstasy users (N=15)	
	Male (N=7)	Female (N=8)	Male (N=9)	Female (N=6)
<b>Demographics</b>				
Age (years)	29.3 ± 6.9	23.3 ± 1.3	25.6 ± 7.5	22.7 ± 2.8
DART-IQ <sup>a</sup>	104.7 ± 6.2	106.9 ± 7.4	111.2 ± 11.5	112.2 ± 8.1
<b>Ecstasy use</b>				
Duration of use (years)	NA	NA	4.6 ± 3.1	3.3 ± 1.5
Usual dose (tablets)	NA	NA	1.33 ± 0.56	1.38 ± 0.49
Lifetime dose (tablets) <sup>c</sup>	NA	NA	29.5 ± 17.5	27.3 ± 19.7
Time since last tablet (months)	NA	NA	4.3 ± 7.5	2.7 ± 2.1
Age of onset (years)	NA	NA	21.3 ± 6.1	19.3 ± 3.1
<b>Other substances</b>				
Alcohol (no. of consumptions/week)	14.1 ± 12.8	7.1 ± 7.4	18.2 ± 14.8	5.3 ± 3.2
Tobacco (cigarettes/day)	9.5 ± 3.3	10.3 ± 6.1	11.0 ± 6.5	9.4 ± 9.2
<i>Last 3 months use of:</i>				
Cannabis (no. of joints) <sup>c</sup>	2.3 ± 0.6	4.5 ± 5.0	68.1 ± 6.5	31.8 ± 51.6
Amphetamine (no. of times used) <sup>c</sup>	-	-	0.4 ± 0.8	-
Usual dose amphetamine (g)	-	-	0.3 ± 0.2	0.1 ± 0.1
Cocaine <sup>c</sup>	-	-	1.2 ± 1.1	-

<sup>a</sup> DART = Dutch Adult Reading Test

<sup>b</sup> Two way analysis of variance

<sup>c</sup> Variables that were log transformed

<sup>d</sup> Statistical significant differences

parametric Kruskal Wallis test, because BDI values were not normally distributed. Posthoc, Mann-Whitney U tests were performed to analyze differences in BDI scores between groups. The correlations between CIDI and BDI outcomes and lifetime ecstasy consumption (log transformed) were analyzed using Spearman's correlation coefficient. A mixed linear model was used to analyze the relationship between CIDI and BDI outcomes and SERT densities. Using this model, it was possible to analyse SPECT data from the six different brain regions studied simultaneously, taking into account both within-subject and between-subject variations. The model we designed included brain regions (6 levels), groups (4 levels), gender (2 levels), and the interaction between group and gender. Outcomes of the CIDI lifetime (2 levels) and BDI (2 levels, cut-off point = median) were added to the model to determine their effects on SERT density.

Two-sided *p*-values below 0.05 were considered to be statistically significant.

Heavy ecstasy users (N=23)		Former ecstasy users (N=16)		<i>p</i> <sub>group</sub> <sup>b</sup>	<i>p</i> <sub>gender</sub> <sup>b</sup>
Male (N=12)	Female (N=11)	Male (N=8)	Female (N=8)		
27.1 ± 6.0	25.0 ± 4.1	26.4 ± 6.2	24.1 ± 4.7	0.63	0.02 <sup>d</sup>
106.0 ± 9.0	104.5 ± 8.4	105.9 ± 11.8	102.0 ± 7.7	0.10	0.73
6.4 ± 3.0	4.6 ± 2.1	4.0 ± 2.0	5.1 ± 3.1	0.24	0.38
2.64 ± 0.67	1.82 ± 0.46	2.00 ± 0.96	2.16 ± 1.01	0.00 <sup>d</sup>	0.35
831.8 ± 733.0	200.9 ± 171.2	126.9 ± 91.4	409.3 ± 868.7	0.00 <sup>d</sup>	0.25
1.97 ± 2.67	2.6 ± 2.1	37.1 ± 25.4	21.0 ± 10.1	0.00 <sup>d</sup>	0.38
20.9 ± 4.2	20.4 ± 3.1	22.4 ± 5.6	19.0 ± 5.0	0.99	0.16
13.0 ± 8.2	5.8 ± 3.5	4.5 ± 3.9	7.9 ± 5.4	0.14	0.00 <sup>d</sup>
12.4 ± 13.0	6.0 ± 7.1	11.8 ± 8.5	13.3 ± 8.6	0.47	0.21
94.6 ± 153.0	67.5 ± 101.9	73.1 ± 110.4	196.3 ± 369.3	0.37	0.23
3.8 ± 7.4	3.6 ± 5.5	-	-	0.04 <sup>d</sup>	0.80
0.4 ± 0.3	0.3 ± 0.3	0.7 ± 0.4	1.0 ± 0.8	0.07	0.83
4.2 ± 2.8	4.4 ± 3.4	-	-	0.09	0.74

## RESULTS

### Characteristics of the study population

Sample characteristics were described in an earlier publication <sup>4</sup>. The four groups were comparable regarding age, gender, premorbid IQ (NART IQ), and use of alcohol and cannabis (Table 1). Ecstasy users had used significantly more amphetamine and cocaine than controls. Males were significantly older (on average 3.1 years) and consumed significantly more alcohol than females. Within the subgroup of heavy ecstasy users, males had used more ecstasy tablets and took higher usual doses than females (Table 1), even when corrected for body weight. Moderate and heavy ecstasy users were abstinent from ecstasy use on average for 3.6 and 2.3 months respectively, while the former heavy ecstasy users reported to be abstinent from ecstasy for almost 2.5 years on average (Table 1).

### CIDI

The prevalence of current or lifetime mood disorders according to DSM-IV criteria measured by CIDI is listed in Table 2. The groups did not differ significantly as to prevalence of lifetime ( $p = 0.930$ ) and current ( $p = 0.866$ ) mood disorders. The overall analysis showed that the prevalence of lifetime mood disorders was not significantly related to gender ( $p = 0.203$ ). Within the ecstasy groups, 12 out of 17 persons (71%) diagnosed with a lifetime mood disorder reported that the onset of their mood disorder had preceded the first use of ecstasy.

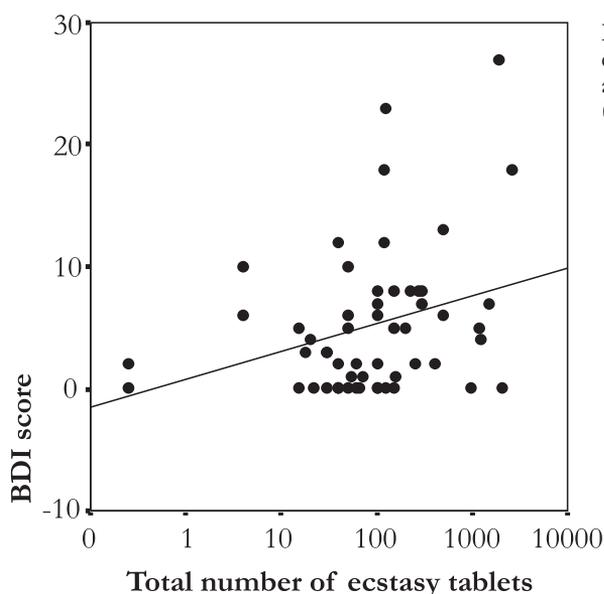
**Table 2:** Mood disorders: frequencies of CIDI lifetime and current mood disorders (DSM-IV) and BDI scores

	Polydrug controls ( $N=15$ )	Moderate ecstasy users ( $N=15$ )	Heavy ecstasy users ( $N=23$ )	Former ecstasy users ( $N=16$ )	
CIDI lifetime mood disorders – no. (%)	4 (27%)	5 (33%)	7 (30%)	6 (38%)	$p_{\text{group}}=0.930$
	2m: 29%, 2f: 25%	1m: 11%, 4f: 67%	5m: 42%, 2f: 18%	1m: 13%, 5f: 63%	$p_{\text{gender}}=0.203$
CIDI current mood disorder – no. (%)	2 (13%)	3 (20%)	4 (17%)	4 (25%)	$p_{\text{group}}=0.866$
	0m: 0%, 2f: 25%	1m: 11%, 2f: 33%	2m: 17%, 2f: 18%	1m: 13%, 3f: 38%	
BDI median score (IRQ: 25-75%)	1.0 (0.0-3.0) <sup>a</sup>	2.0 (0.0-4.0)	5.0 (0.0-8.0)	5.5 (1.3-10.0)	$p_{\text{group}}=0.056$
	m=1.5, f=1.0	m=0.0, f=3.5	m=4.5, f=6.0	m=4.0, f=7.5	$p_{\text{gender}}=0.179$

<sup>a</sup> Two subjects with missing data

## BDI

Results of the BDI are also listed in Table 2. Two controls did not complete the BDI questionnaire, so only the results of 13 controls are presented. The overall test for differences in BDI scores between groups was near significance ( $p = 0.056$ ). Post-hoc, between-group comparisons revealed significantly higher BDI scores in former heavy ecstasy users ( $p = 0.045$ ) than in ecstasy-naïve polydrug controls. BDI scores correlated significantly ( $r = 0.310$ ,  $p = 0.021$ ) with the log-transformed total number of ecstasy tablets (Figure 1). BDI scores did not differ between men and women ( $p = 0.179$ ). No significant correlations were found between BDI scores and the usual dose of ecstasy per event ( $r = 0.144$ ,  $p = 0.303$ ) or between BDI scores and the use of cannabis in the last year ( $r = 0.213$ ,  $p = 0.182$ ).



**Figure 1:** Correlation between depressive symptoms (BDI scores) and lifetime ecstasy consumption (logarithmic scaling)

## Mood and SERT

Mean SERT densities are shown in Table 3, sub-divided for subjects with and without a diagnosis of lifetime mood disorder (CIDI). In addition, mean SERT densities are given, subdivided for BDI scores above or below the median value (median value=3.0). As described in an earlier publication<sup>4</sup>, a significant group effect was observed ( $p = 0.041$ ) in overall [<sup>123</sup>I]β-CIT binding ratios, in which overall SERT densities appeared to be lower in the group of heavy ecstasy users than in the other groups. Moreover, a significant interaction between group and gender was observed, in which overall SERT densities in female ecstasy users appeared to be lower than in male ecstasy

**Table 3:** Mean overall [<sup>123</sup>I]β-CIT binding ratios (±SD) for persons with/without lifetime mood disorder (CIDI) and BDI score above/below median score

	Polydrug controls	Moderate ecstasy users	Heavy ecstasy users	Former ecstasy users	Main effect on SERT
CIDI: with lifetime mood disorder	1.31 ± 0.12	1.18 ± 0.14	1.14 ± 0.12	1.23 ± 0.06	CIDI: $p=0.995$
without lifetime mood disorder	1.21 ± 0.09	1.16 ± 0.11	1.16 ± 0.11	1.23 ± 0.12	
BDI: above median BDI score (BDI ≥3)	1.22 ± 0.04	1.18 ± 0.11	1.13 ± 0.10	1.24 ± 0.09	BDI: $p=0.410$
below median BDI score (BDI <3)	1.24 ± 0.12	1.19 ± 0.14	1.19 ± 0.11	1.22 ± 0.12	

users. However, we observed no main effect of CIDI ( $p = 0.995$ ) or BDI ( $p = 0.410$ ) on SERT when these variables were added to our statistical model.

## DISCUSSION

The present study investigated the short-term as well as the long-term effects of ecstasy on mood and its association with serotonergic neurotoxicity. We detected no significant effect of ecstasy use on the prevalence of CIDI-DSM-IV mood disorders. However, we found significantly more depressive symptoms (higher BDI scores) in former heavy ecstasy users than in controls that used different drugs but no ecstasy. The BDI scores also correlated with the number of ecstasy tablets used. We observed no association between mood disorders and SERT densities or gender.

The increased symptoms of depression (measured using BDI) might be caused by deficits in serotonin in (former) heavy ecstasy users, as there is evidence that ecstasy has neurotoxic effects on the serotonin system and as it is known that the serotonin system modulates many psychological functions. Because the number of participants in each group was relatively small, we cannot fully exclude the possibility of a chance finding, as indicated by the overall  $p$ -value for the difference between groups ( $p = 0.056$ ). However, other research groups have also reported more symptoms of depression in recent and former ecstasy users<sup>19,20,22-25,30</sup>. However, only one previous study examined the long-term effect of ecstasy use on mood in subjects that stopped using ecstasy for one year<sup>24</sup> and found elevated depression scores in these former ecstasy users. The observation of increased depressive symptoms in former heavy ecstasy users in the present study corroborates this finding.

Using DSM-IV criteria (CIDI), we observed no difference in prevalence of current or lifetime mood disorders between ecstasy users and controls. Because CIDI gives a binary clinical diagnosis (mood disorder vs. no mood disorder), the statistical power of this study might have been too low to demonstrate increased prevalence of clinical mood disorders in (former) ecstasy users. BDI, on the other hand, is more sensitive

because of its gradual scale including both sub-clinical and clinical scores. BDI scores from 0 to 9 are considered to be within the normal range. Therefore, median BDI scores within all four subgroups ranging from 1.0 to 5.5 (Table 2) did not reach the clinical level of depression.

A relationship between depressive symptoms and decreased SERT availability has been reported in several diseases, such as major depression<sup>41</sup>, seasonal affective disorders<sup>42</sup>, and Wilson's disease<sup>43</sup>. On the other hand, Dahlström *et al.*<sup>44</sup> demonstrated elevated SERT density in depressive children and adolescents. In the present study, we found no correlation between overall SERT binding and mood disorders as measured by CIDI or BDI. Interestingly, when comparing former ecstasy users with current heavy ecstasy users, it seems that BDI scores do not improve. This is in remarkable contrast to the reversibility of decreased SERT binding, observed in former (female) heavy ecstasy users<sup>4,5</sup>. On the other hand, this finding is in agreement with findings of Thomasius *et al.*<sup>30</sup> who observed reduced SERT binding only in current but not in former ecstasy users, while elevated depressive symptoms were reported by both current and former ecstasy users and not by ecstasy-naïve polydrug controls. Although no association between depressive symptoms and SERT density was calculated, this would suggest that the observed depressive symptoms were not correlated to serotonergic neurotoxicity induced by ecstasy. We, as well as others, previously reported a similar lack of association between SERT densities and memory function<sup>2,5</sup>.

There are several possible explanations why we did not observe a correlation between mood and SERT densities. The first explanation is that depressive symptoms in (former) ecstasy users are not attributable to serotonin deficits induced by ecstasy. This is in concordance with our observation and with the observation of Lieb *et al.*<sup>26</sup> that the majority of (former) ecstasy users with mood disorders reported the onset of their mood disorder preceding the first use of ecstasy. On the other hand, we observed a modest but significant correlation between the extent of previous ecstasy consumption and symptoms of depression, similar to the one described by Thomasius *et al.*<sup>30</sup>, which suggests that increased depressive symptoms might be at least partially attributable to ecstasy use. A second explanation therefore might be that, although loss of SERT is indicative of axonal loss or damage, recovery of SERT densities does not imply normal (behavioural) function. Possibly in line with this, Hatzidimitriou *et al.*<sup>45</sup> showed that after administration of neurotoxic doses of MDMA in non-human primates partial recovery of brain serotonin axons was accompanied by development of altered reinnervation patterns. A final explanation might be that the radiotracer [<sup>123</sup>I]β-CIT used in this study has a limited sensitivity in detecting serotonergic neurotoxicity because it is not selective for binding to SERTs. The use of a selective radiotracer for SERTs might be more sensitive and therefore more capable of detecting a possible correlation between mood and SERT densities.

We found no correlation between gender and prevalence of lifetime mood disorders (CIDI) or BDI scores, whereas earlier findings in the same study population suggested that females are more vulnerable to the effects of ecstasy on SERT densities than males <sup>4</sup>. It was also reported elsewhere that females had a more pronounced subjective response to ecstasy and were more susceptible to mid-week low mood <sup>17,31</sup>. Moreover, mood disorders in the Netherlands were reported to be almost twice as common among females as among males <sup>46</sup>. Probably because of the low prevalence of clinical mood disorders within all subgroups, we did not observe overall significant differences in mood between males and females.

The relatively small groups and the low prevalence of clinical mood disorders within the groups resulted in limited statistical power of this study, especially with regard to the control of and adjustment for potential confounders. However, except for the lifetime number of tablets, we found no significant correlations between BDI scores and other characteristics of ecstasy use, such as duration of use, usual dose, time since last tablet, and age of onset of ecstasy use. In addition, we did not find any significant correlation between BDI scores and other potential confounders, such as use of alcohol, tobacco, cannabis, amphetamine or cocaine. We therefore believe that it is unlikely that these factors confounded our results, but because of the limited statistical power of this study a potential influence cannot be entirely excluded.

In conclusion, our findings suggest that symptoms of depression in ecstasy users are related to the number of ecstasy tablets used. Moreover, our findings suggest that symptoms of depression may still be present more than one year after ecstasy use is stopped. When a clinical depression is ecstasy-related, involving the serotonergic neurotransmitter system, this should have implications for treatment. As suggested by Haddad *et al.* <sup>47</sup>, in that case treatment with norepinephrine reuptake inhibitors such as reboxetine, would be preferable over selective serotonin re-uptake inhibitors (SSRIs) such as fluoxetine, since the norepinephrine system is thought to remain intact in ecstasy users. Because this study has limited statistical power, our results need confirmation from larger studies. Moreover as this study was performed retrospectively, and as we found no evidence that mood disorders in ecstasy users are correlated to decreased SERT binding, no final conclusion can be drawn about the causal nature of the observed relationship between ecstasy use and depressed mood. It remains possible that there are pre-existing differences in mood between ecstasy users and controls. In order to establish a causal relationship prospective studies are in progress.

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CHAPTER

9

# **Memory Function and Serotonin Transporter Promoter Gene Polymorphism in Ecstasy (MDMA) Users**

Journal of Psychopharmacology 2006; 20: 389-399

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## ABSTRACT

### Background

Although 3,4-methylenedioxymethamphetamine (MDMA or ecstasy) has been shown to damage brain serotonin (5-HT) neurons in animals and possibly humans, little is known about the long-term consequences of ecstasy-induced serotonin neurotoxic lesions on functions in which serotonin is involved, such as cognitive function. Because serotonin transporters play a key element in the regulation of synaptic serotonin transmission it may be important to control for the potential covariance effect of a polymorphism in the serotonin transporter promoter gene region (5-HTTLPR) when studying the effects of ecstasy as well as cognitive functioning.

### Aim

To investigate the effects of moderate and heavy ecstasy use on cognitive function, as well as the effects of long-term abstinence from ecstasy, in subjects genotyped for 5-HTTLPR. A second aim of the study was to determine whether these effects differ for females and males.

### Material and Methods

Fifteen moderate ecstasy users (< 55 lifetime tablets), 22 heavy ecstasy users (> 55 lifetime tablets), 16 ex-ecstasy users (last tablet > 1 year ago) and 13 controls were compared on a battery of neuropsychological tests. DNA from peripheral nuclear blood cells was genotyped for 5-HTTLPR using standard polymerase chain reaction methods.

### Results

A significant group effect was observed only on memory function tasks ( $p = 0.04$ ) but not on reaction times ( $p = 0.61$ ) or attention/ executive functioning ( $p = 0.59$ ). Heavy and ex-ecstasy users performed significantly poorer on memory tasks than controls. In contrast, no evidence of memory impairment was observed in moderate ecstasy users. No significant effect of 5-HTTLPR or gender was observed.

### Conclusions

The results mean that while the use of ecstasy in quantities that may be considered 'moderate' is not associated with impaired memory functioning, heavy use of ecstasy may lead to long lasting memory impairments.

## INTRODUCTION

Though generally regarded as relatively safe, it has become increasingly apparent that the popular recreational drug 3,4-methylenedioxymethamphetamine (MDMA or ecstasy) can lead to toxic effects on brain serotonin (5-HT) neurons in animals and possibly humans<sup>1-5</sup>. In animals, damage to serotonin neurons has been demonstrated by reductions in various markers unique to serotonergic axons, including the density of serotonin transporters<sup>6-9</sup>. Since ecstasy-induced serotonergic neurotoxic damage may lead to impairment of functions in which serotonin is involved (e.g. memory function)<sup>10-12</sup> it is not only important to study the effects of ecstasy on serotonergic neurons, but on cognitive function as well. Memory function is of particular interest since several studies have found that ecstasy users display significant memory impairments, whereas their performance on other cognitive tests is generally normal<sup>13-15</sup>. In animals, ecstasy severely damages serotonergic axons in brain regions involved in memory function, including the hippocampus and cerebral cortex<sup>16,17</sup>.

While the short-term neurotoxic effects of ecstasy on serotonergic neurons and memory have been studied extensively, little is known about the long-term effects in humans. Studies in nonhuman primates have shown that up to 7 years after treatment with MDMA neocortical brain regions remain partially denervated, while other regions show evidence of complete recovery<sup>18</sup>. Furthermore, it is unclear, whether moderate use of ecstasy can produce these changes.

There is some evidence suggesting that females have increased susceptibility to psychological and neurotoxic effects of ecstasy. Higher depression and anxiety scores have been observed in female ecstasy users when compared to male users<sup>19,20</sup>. Greater reductions in cerebrospinal fluid 5-hydroxyindoleacetic acid (5-HIAA) and serotonin transporter densities have been observed in female ecstasy users compared to males<sup>2,21,22</sup>.

Several studies suggest that serotonin transporters may play an important role in cognitive processes, such as memory function<sup>23</sup>. As a key element in the regulation of synaptic transmission in serotonergic neurons, the serotonin transporter has become an important research target. For instance, it has been shown that selective serotonin reuptake inhibitors in non-demented elderly depressed patients improved both mood and cognitive function<sup>24</sup>. Recently, a polymorphism in the serotonin transporter promoter gene region (5-HTTLPR<sup>25</sup>) has been shown to regulate serotonin transporter density in human cell lines<sup>26</sup>. Besides reduced serotonin transporter expression, the *in vitro* transcriptionally less active 5-HTTLPR *s* allele has been associated with depression and anxiety-related personality traits<sup>26,27</sup>. In line with this, Heinz and colleagues<sup>28</sup> found reduced *in vivo* serotonin transporter densities (as measured with [<sup>123</sup>I]β-CIT SPECT) in healthy subjects carrying the *s* allele, although others have not observed such an association<sup>29</sup>. There are also implications that the

5-HTTLPR genotype affects serotonin transporter availability in disease<sup>30</sup>. It is also conceivable that the 5-HTTLPR genotype modulates neurotoxic effects of drugs, such as ecstasy, on the serotonin system<sup>31</sup>. It has been argued that low serotonin function may be a cause rather than an effect of ecstasy use, since low serotonin levels have been linked to impaired cognitive functioning and impulsivity or sensation seeking in humans<sup>32</sup>. Based on these considerations, it could be hypothesized that the *s* allele is associated with ecstasy use and/or cognitive function, and therefore an important confounding variable when investigating cognitive function in users of this drug. Furthermore, it has recently been suggested that the 5-HTTLPR genotype mediates emotionally related cognitive disturbance in ecstasy users, because ecstasy users carrying the *s* allele, and not comparison subjects carrying the *s* allele, showed abnormal emotional processing<sup>33</sup>.

The present study investigated a positive association between ecstasy dose and cognitive function in subjects genotyped for 5-HTTLPR. Furthermore, the effects of long-term abstinence from ecstasy use were analysed, as well as the effects of gender on cognitive function. We hypothesized that we would observe: (1) a negative and dose related effect of ecstasy on memory function and no effect on the other cognitive domains studied, (2) no difference between memory impairment of heavy users and ex-users (3) an association between the *s* allele and ecstasy use and/or memory impairment, and (4) greater impairment in memory function in female ecstasy users than in males.

A part of this study concerning the Rey Auditory Verbal Learning Test (RAVLT) in heavy ecstasy users has been previously published<sup>3</sup>. In addition, in three other publications we reported on serotonin and dopamine transporter densities and mood disorders in the same or a subset of subjects as in the present analysis<sup>2,34,35</sup>. However, in the present study the effects of ecstasy on other memory tests and other cognitive domains of that population are presented, as well as possible confounding thereof by gender and 5-HTTLPR. In addition, the present analysis includes moderate ecstasy users in order to investigate in more detail the dose related effects of ecstasy on cognitive function.

## MATERIAL AND METHODS

### Participants

Recruitment of the participants was as previously described<sup>2,3,34,35</sup>. Briefly, three different groups of ecstasy users were compared with ecstasy-naive but drug-using controls. Subjects were recruited with flyers distributed at venues associated with the 'rave scene' in Amsterdam with the help of Unity, an agency which provides

harm reduction information and advice. Experimental and control groups were thus recruited from the same community sources. Subjects selected were group matched for gender and age, between 18 and 45 years, otherwise healthy, and with no psychiatric history. Three different groups of ecstasy users were recruited: 15 moderate ecstasy users ('ecstasy group'), 22 heavy ecstasy users ('heavy ecstasy group'), and 16 former heavy ecstasy users ('former ecstasy group'). The eligibility criterion for the moderate ecstasy group was previous use of maximum 50 tablets of ecstasy, whereas the heavy ecstasy group had to have used at least 50 tablets prior to the study. The former ecstasy group had to have taken a minimum of 50 tablets but stopped using ecstasy for at least 1 year prior to the study. The cut-off 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 ecstasy tablets<sup>36</sup>. The 13 controls were healthy subjects with no self-reported prior use of ecstasy.

Participants agreed to abstain from use of all psychoactive drugs for at least 3 weeks before the study, and were asked to undergo urine drug screening on the day of the study (with an enzyme-multiplied immunoassay for amphetamines, barbiturates, benzodiazepine metabolites, cocaine metabolite, opiates, and cannabis) before enrolment. Subjects were interviewed with a fully structured computer assisted diagnostic psychiatric interview (Composite International Diagnostic Interview: CIDI, version 2.1) to screen for current axis I psychiatric diagnoses. After testing urine samples, exclusion criteria were: a positive drug screen (5 subjects were excluded); pregnancy; and a severe medical or neuropsychiatric illness that precluded informed consent.

Subjects were informed that reimbursement for participation was contingent on no evidence of drug use on the urine sample. The institutional Medical Ethics Committee approved the study. After complete description of the study to the subjects, written informed consent was obtained from all participants.

## Neuropsychological testing

We selected a battery of widely used tests that have been related to serotonergic functions, particularly memory<sup>37</sup>.

### Test of general intelligence

Dutch Adult Reading Test (DART)<sup>38</sup>. Fifty words with irregular spelling are read aloud. The number of correctly read words is transformed into an estimate of verbal intelligence (DART-IQ). The DART is the Dutch counterpart of the National Adult Reading Test (NART)<sup>39</sup>. This test gives an estimate of premorbid intelligence, as it is relatively insensitive to cognitive deterioration due to neurological disorders. It was used to describe the sample and as a covariate in the statistical analyses.

## Reaction speeds

Reaction was tested using FePsy, an automated computerized battery of validated neuropsychological tasks<sup>40</sup>. Reaction was evaluated separately on the non-dominant hand and dominant hand in response to simple auditive and visual stimuli, and to a Binary Choice Task.

## Memory function

- *Logical Memory of the Rivermead Behavioural Memory Test*<sup>41</sup>. A 21-item news message is read to the subject, who repeats as many items as he or she can remember. After a 15-minute interval he or she is asked to recall the message again. Score is the number of items recalled. In view of the limited reliability of this type of test, two messages were used and the scores were summed.
- *Visual Reproduction subtest of the Wechsler Memory Scale – Revised (WMS-R)*<sup>42</sup>. Four geometric figures are shown to the subject, one by one during 10 seconds. Immediately after presentation the subject draws each figure from memory. After a delay of 30 minutes he is asked to draw the figures once again. The number of correctly reproduced elements is scored. Total scores range to a maximum of 41 points.
- *Rey Auditory Verbal Learning Test (RAVLT)*<sup>43</sup>. The subject memorizes a series of 15 words in five learning trials. Following a 20-minute delay, the subject is asked to recall the word list. Raw scores are used.
- *Corsi Block-tapping Test*<sup>44</sup>. This is a test of spatial memory span. The subject has to reproduce a series of taps on blocks that are randomly dispersed on a board. The length of the series is gradually increased until the subject consistently fails.

## Tests of attention and executive functioning

- *Category fluency*<sup>45</sup>. Naming animals and occupations, for 1 minute each. Score is raw number correct in 2 minutes.
- *Controlled Oral Word Association Test (COWAT)*<sup>46</sup>. During 1 minute the subject must say as many words as he or she can think of that begin with a given letter. Three trials with different letters were done. Score is raw number correct in 3 minutes.
- *Stroop Color Word Test*<sup>47,48</sup>. This test measures perceptual interference, response inhibition, and selective attention by having subject name colours, and name the colour of ink of colour-words when the words are printed in a non-matching coloured ink. Score is the time to completion in seconds for 100 items.
- *Trail Making Test part A and B*<sup>49,50</sup>. The task is to connect numbers (part A) and to connect numbers alternating with letters (part B) on a sheet of paper. This is a test of visual scanning, visuomotor and conceptual tracking, mental flexibility, and motor speed. Score is time to completion in seconds.

- *Wisconsin Card Sorting Test* (WCST) <sup>51</sup>. This test uses a deck of cards on which different numbers of different forms in different colours are shown. The task is to sort the cards according to one of three possible sorting rules (colour, number, or form). These rules are not told to the subject; he or she must identify the sorting rules. However, after each sort feedback is given on whether it was correct. Once a sorting rule has been found (ten correct sorts on a row), the sorting rule is changed without warning, so that the subject has to shift to a different rule. Of particular interest are perseverative errors of the kind where the subject keeps sorting according to a previously correct rule or to a rule that he or she was told to be wrong in the immediately preceding sort. The WCST is a test of concept formation and set shifting. Scores are the raw numbers of errors, perseverations and sort shifts ('categories').

## Genotyping

Genotyping was performed using peripheral nuclear cells obtained by centrifugation of approximately 5 ml blood from the antecubital vein. 5-HTTLPR *l* and *s* alleles were analysed using polymerase chain reaction as described elsewhere <sup>25,26</sup>.

## Statistical analyses

### Characteristics of the sample

Differences in continuous variables (log transformed if necessary) between the four groups were analysed using ANOVA and Bonferroni *post hoc* analysis. Differences in the prevalence of subjects carrying the *s* allele between ecstasy users and control subjects were investigated using the Chi-square test. In addition, Pearson correlation analysis was performed between the number of *s*-allele 5-HTTLPR genotype and extent of previous ecstasy use.

### Neuropsychological testing

Differences between the four groups in the three main cognitive domains (reaction speed, memory function and attention/executive functioning) were analysed using general linear model-based MANOVA. To answer our research question, our basic model included a cognitive domain (reaction speed six levels; memory function eight levels and attention/executive functioning nine levels), group (four levels), 5-HTTLPR (three levels) and gender (two levels), and the interaction between group and 5-HTTLPR, and group and gender. We extended the model by including several potential confounders, including age (continuous), DART-IQ (continuous), extent of previous cannabis use (continuous). If a significant confounding effect was observed, the variable was kept in the model. If MANOVA revealed a significant group effect,

we investigated differences in cognitive parameters between groups by one-way ANOVA and Bonferroni *post hoc* analysis.

Correlations between cognitive parameters (on which the four groups differed significantly) and extent of previous ecstasy use were assessed using Pearson correlation analyses. Because age, gender, verbal intelligence and extent of previous cannabis use have been shown to be highly associated with the majority of memory tests, we also performed partial correlations to control for age, gender, DART-IQ and extent of previous cannabis use. In addition, partial correlations were assessed between cognitive parameters and extent of previous cannabis use while controlling for age, gender, DART-IQ and extent of previous ecstasy use.

The chance of a type I error ( $\alpha$ ) was set at 0.05. In case Bonferroni *post hoc* analyses were made, statistical significance within the text will be reported as a corrected *p* value. All data were analysed using SPSS version 10.0 (SPSS, Inc., Chicago, USA).

**Table 1:** Demographics, characteristics of ecstasy use and exposure to other substances expressed as mean  $\pm$  SD

	Polydrug controls (N=15)		Moderate ecstasy users (N=15)	
	Male (N=7)	Female (N=8)	Male (N=9)	Female (N=6)
<b>Demographics</b>				
Age (years)	29.3 $\pm$ 6.9	23.3 $\pm$ 1.3	25.6 $\pm$ 7.5	22.7 $\pm$ 2.8
DART-IQ <sup>a</sup>	104.7 $\pm$ 6.2	106.9 $\pm$ 7.4	111.2 $\pm$ 11.5	112.2 $\pm$ 8.1
<b>Ecstasy use</b>				
Duration of use (years)	NA	NA	4.6 $\pm$ 3.1	3.3 $\pm$ 1.5
Usual dose (tablets)	NA	NA	1.33 $\pm$ 0.56	1.38 $\pm$ 0.49
Lifetime dose (tablets) <sup>c</sup>	NA	NA	29.5 $\pm$ 17.5	27.3 $\pm$ 19.7
Time since last tablet (months)	NA	NA	4.3 $\pm$ 7.5	2.7 $\pm$ 2.1
<b>Other substances</b>				
Alcohol (no.of consumptions/week)	14.1 $\pm$ 12.8	7.1 $\pm$ 7.4	18.2 $\pm$ 14.8	5.3 $\pm$ 3.2
Tobacco (cigarettes/day)	9.5 $\pm$ 3.3	10.3 $\pm$ 6.1	11.0 $\pm$ 6.5	9.4 $\pm$ 9.2
<i>Last 3 months use of:</i>				
Cannabis (no. of joints) <sup>c</sup>	2.3 $\pm$ 0.6	4.5 $\pm$ 5.0	68.1 $\pm$ 6.5	31.8 $\pm$ 51.6
Amphetamine (no. of times used) <sup>c</sup>	-	-	0.4 $\pm$ 0.8	-
Usual dose amphetamine (g)	-	-	0.3 $\pm$ 0.2	0.1 $\pm$ 0.1
Cocaine <sup>c</sup>	-	-	1.2 $\pm$ 1.1	-

<sup>a</sup> DART = Dutch Adult Reading Test

<sup>b</sup> Two way analysis of variance

<sup>c</sup> Variables that were log transformed

<sup>d</sup> Statistical significant differences

## RESULTS

### Characteristics of the sample

Characteristics of the study population were described in an earlier publication<sup>3</sup>. The four different groups were comparable regarding age, sex distribution, premorbid IQ (NART IQ), and use of alcohol and cannabis (Table 1). Ecstasy users had used significantly more amphetamine than controls. Males were significantly older (on average 3.1 years) and consumed significantly more alcohol per week than females. Within the heavy ecstasy subgroup, males had used more ecstasy tablets during lifetime and higher usual doses than females (Table 1), also when expressed per kg body weight. On average, moderate and heavy ecstasy users were abstinent from ecstasy use for 3.6 and 2.3 months respectively, while the former ecstasy users reported to be abstinent from ecstasy for almost 2.5 years on average (Table 1).

Heavy ecstasy users (N=23)		Former ecstasy users (N=16)		$p_{\text{group}}^b$	$p_{\text{gender}}^d$
Male (N=12)	Female (N=11)	Male (N=8)	Female (N=8)		
27.1 ± 6.0	25.0 ± 4.1	26.4 ± 6.2	24.1 ± 4.7	0.63	0.02 <sup>d</sup>
106.0 ± 9.0	104.5 ± 8.4	105.9 ± 11.8	102.0 ± 7.7	0.10	0.73
6.4 ± 3.0	4.6 ± 2.1	4.0 ± 2.0	5.1 ± 3.1	0.24	0.38
2.64 ± 0.67	1.82 ± 0.46	2.00 ± 0.96	2.16 ± 1.01	0.00 <sup>d</sup>	0.35
831.8 ± 733.0	200.9 ± 171.2	126.9 ± 91.4	409.3 ± 868.7	0.00 <sup>d</sup>	0.25
1.97 ± 2.67	2.6 ± 2.1	37.1 ± 25.4	21.0 ± 10.1	0.00 <sup>d</sup>	0.38
13.0 ± 8.2	5.8 ± 3.5	4.5 ± 3.9	7.9 ± 5.4	0.14	0.00 <sup>d</sup>
12.4 ± 13.0	6.0 ± 7.1	11.8 ± 8.5	13.3 ± 8.6	0.47	0.21
94.6 ± 153.0	67.5 ± 101.9	73.1 ± 110.4	196.3 ± 369.3	0.37	0.23
3.8 ± 7.4	3.6 ± 5.5	-	-	0.04 <sup>d</sup>	0.80
0.4 ± 0.3	0.3 ± 0.3	0.7 ± 0.4	1.0 ± 0.8	0.07	0.83
4.2 ± 2.8	4.4 ± 3.4	-	-	0.09	0.74

## Neuropsychological testing

Table 2 represents the scores on the three main cognitive domains (reaction speed, memory function and attention/executive functioning) analysed. MANOVA only revealed a significant main effect of Group on memory function ( $F = 1.66$ ,  $df = 24$ ,  $p = 0.03$ ), but not on reaction times ( $F = 0.87$ ,  $df = 18$ ,  $p = 0.61$ ) or attention/executive functioning ( $F = 0.92$ ,  $df = 27$ ,  $p = 0.59$ ).

**Table 2:** Reaction times and cognitive performance (memory and attention)\*

	Controls <i>N</i> = 13 <sup>a</sup>	MDMA <i>N</i> = 15	MDMA+ <i>N</i> = 22 <sup>b</sup>	ex-MDMA <i>N</i> = 16	<i>p</i>
<b>Median Reaction Times (msec)</b>					<b>0.61</b>
Auditive DH	242.5 ± 22.1	246.7 ± 28.3	245.2 ± 30.2	244.1 ± 29.3	
Auditive NH	244.4 ± 34.6	250.1 ± 24.1	245.5 ± 26.8	254.3 ± 32.3	
Visual DH	282.1 ± 52.2	287.7 ± 55.2	257.4 ± 30.7	270.3 ± 46.6	
Visual NH	316.0 ± 92.8	298.6 ± 56.2	268.7 ± 31.7	279.9 ± 53.6	
Binary choice task	382.9 ± 112.6	368.2 ± 53.0	353.7 ± 67.9	368.3 ± 69.5	
Binary choice (total falses)	2.5 ± 3.4	2.6 ± 3.1	5.0 ± 7.2	2.6 ± 1.9	
<b>Memory function (total scores)</b>					<b>0.03<sup>†</sup></b>
Logical memory immediate	17.9 ± 6.1	16.1 ± 5.2	17.9 ± 3.8	16.3 ± 5.8	0.15
Logical memory delayed	15.3 ± 5.8	12.7 ± 5.4	14.4 ± 3.9	13.8 ± 6.2	0.12
WMS immediate	39.4 ± 1.9	39.2 ± 1.8	38.4 ± 2.6	37.7 ± 3.2	0.44
WMS delayed	36.4 ± 3.4	36.2 ± 5.5	35.4 ± 5.6	35.9 ± 4.1	0.91
RAVLT immediate	60.0 ± 6.8	51.2 ± 8.6	47.0 ± 8.6	48.0 ± 12.5	0.00 <sup>‡</sup>
RAVLT delayed	13.1 ± 2.1	10.7 ± 3.2	9.8 ± 2.9	10.1 ± 2.9	0.00 <sup>§</sup>
Corsi Block Span	5.2 ± 0.7	5.7 ± 1.1	5.6 ± 1.3	5.7 ± 1.3	0.59
Corsi Block Span plus one	5.6 ± 0.6	5.9 ± 1.0	6.0 ± 1.1	6.0 ± 1.2	0.51
<b>Attention and executive functioning (total scores)</b>					<b>0.59</b>
Category fluency (sum score)	44.3 ± 7.5	47.0 ± 12.6	45.1 ± 7.4	41.4 ± 10.2	
Letterfluency (sum score)	44.4 ± 9.3	41.5 ± 9.8	41.6 ± 12.6	39.6 ± 10.4	
Stroop color (sec)	53.9 ± 9.0	56.7 ± 10.5	53.2 ± 9.0	53.5 ± 7.9	
Stroop color-word (sec)	82.6 ± 14.4	83.5 ± 12.0	82.0 ± 15.5	85.5 ± 14.4	
Trailmaking A (sec)	24.8 ± 7.5	20.6 ± 6.5	19.9 ± 3.7	24.0 ± 11.6	
Trailmaking B (sec)	47.9 ± 12.5	49.7 ± 14.5	46.4 ± 15.7	52.5 ± 13.5	
WCST errors	35.3 ± 24.0	36.7 ± 22.8	38.8 ± 18.3	35.5 ± 19.2	
WCST perseverations	19.3 ± 15.7	15.8 ± 8.5	19.7 ± 14.6	15.1 ± 13.6	
WCST categories	4.6 ± 1.5	4.8 ± 1.7	4.4 ± 1.6	4.7 ± 2.1	

<sup>a</sup> Two subjects missing with data; <sup>b</sup> one subject missing with data

\* Data are expressed in mean ± SD

<sup>†</sup> Significant group effect ± MANOVA:  $F_{24} = 1.66$

<sup>‡</sup> *Post hoc* analysis: Control vs MDMA group, corrected  $p = 0.11$ , control vs MDMA+ group, Bonferroni corrected  $p = 0.00$ , control vs ex-MDMA group, Bonferroni corrected  $p = 0.01$ .

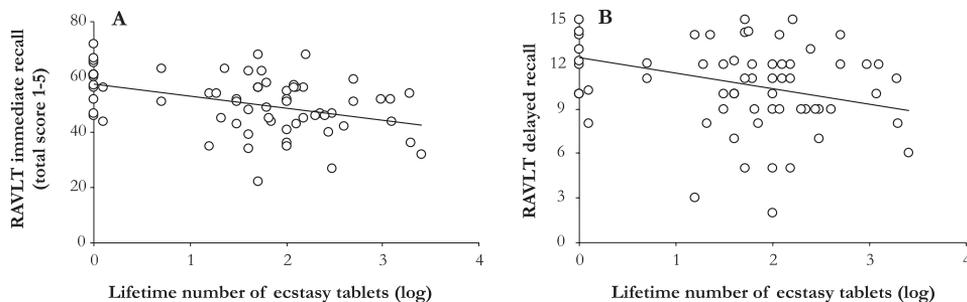
<sup>§</sup> *Post hoc* analysis: Control vs MDMA group, corrected  $p = 0.20$ , control vs MDMA+ group, Bonferroni corrected  $p = 0.01$ , control vs ex-MDMA group, Bonferroni corrected  $p = 0.04$ .

## Memory function

Univariate ANOVA demonstrated a significant group effect on RAVLT immediate ( $F = 7.1$ ,  $df = 3$ ,  $p < 0.01$ ) and delayed word recall ( $F = 5.6$ ,  $df = 3$ ,  $p = 0.00$ ). *Post hoc* analysis showed that heavy ( $p < 0.01$ ), but not moderate ( $p = 0.11$ ) ecstasy users recalled significantly less words when compared to controls. Former ecstasy users also recalled significantly less words on the immediate RAVLT when compared to controls. Similar observations were made on the delayed RAVLT recall: heavy and former ecstasy users recalled less words ( $p = 0.01$  and  $p = 0.04$ , respectively) as compared to controls, but not moderate ecstasy users ( $p = 0.20$ ).

When analysing memory function, MANOVA revealed no significant effect of the between-group factors of 5-HTTLPR genotype ( $p = 0.76$ ) and gender ( $p = 0.13$ ). In addition, no significant interactions were observed between group and 5-HTTLPR ( $p = 0.74$ ), and group and gender ( $p = 0.82$ ). No significant covariance effect of extent of previous cannabis use (log transformed;  $p = 0.21$ ) was observed. However, a significant effect of DART-IQ ( $p < 0.01$ ) and age ( $p = 0.03$ ) was observed, and within groups comparisons were thus controlled for these two variables.

In the whole sample, extent of previous ecstasy use (lifetime number amount of tablets; log-transformed) was significantly associated with immediate ( $r = -0.42$ ,  $p < 0.01$ ) and delayed RAVLT scores ( $r = 0.33$ ,  $p = 0.01$ ) (Figure 1). However, no significant correlations within just the three ecstasy using groups were observed, suggesting that although there is a general difference between ecstasy users and non-users, this difference may not be attributable to amount of use *per se*. When controlling for potential confounders (age, gender, DART-IQ, and extent of previous cannabis use) in the partial correlation analysis, the associations between extent of previous ecstasy use and RAVLT scores remained significant ( $r = -0.36$ ,  $df = 61$ ,  $p < 0.01$ , and  $r = -0.28$ ,  $df = 60$ ,  $p = 0.03$ , respectively) in the whole sample. Extent of previous cannabis use (number of joints in last 3 months; log-transformed) was significantly associated with immediate ( $r = -0.26$ ,  $p = 0.04$ ), but not delayed RAVLT scores ( $r =$



**Figure 1** **A.** Correlation between RAVLT immediate recall scores and extent of previous ecstasy use (log-transformed). **B.** Correlation between RAVLT delayed recall scores and extent of previous ecstasy use (log-transformed).

-0.11,  $p = 0.38$ ). However, when controlling for age, gender, DART-IQ, and extent of previous ecstasy use, the observed association between extent of previous cannabis use and immediate recall did not remain significant (log-transformed;  $r = -0.14$ ,  $df = 61$ ,  $p = 0.27$ ), nor the delayed recall ( $r = -0.02$ ,  $df = 60$ ,  $p = 0.88$ ).

## Genotype

Genotype distribution in ecstasy users was in good accordance with 5-HTTLPR genotype distribution patterns found in healthy white European subjects <sup>26</sup>: *ll*, 17 (32.1%); *lls*, 28 (52.8%); *ss*, eight (15.1%). Controls and ecstasy users did not differ in genotype distribution patterns ( $X^2$ ,  $p = 0.38$ ). MANOVA demonstrated no significant effect of genotype on memory function ( $p = 0.76$ ), nor an interaction between memory function and genotype ( $p = 0.74$ ). Finally, correlation analysis demonstrated no significant relationships between the 5-HTTLPR genotype and extent of previous ecstasy use ( $p = 0.71$ ). Thus, as also shown above, the 5-HTTLPR genotype is not related to performance on the memory tests and is not a confounder of the observed relation between ecstasy use and memory performance.

## DISCUSSION

Our findings indicate impairments in memory function in heavy users of ecstasy with relatively intact performance in reaction times and tasks of attention and executive functioning. Similar observations were made in individuals who stopped using ecstasy more than 1 year ago. In contrast, no evidence of cognitive impairment was observed in subjects who indicated having used ecstasy in quantities that may be considered 'moderate'. Last, our preliminary data provide no evidence for a role of 5-HTTLPR genotype in ecstasy (ab)use or cognitive performance, nor gender differences in susceptibility to ecstasy-induced memory impairment.

Interestingly, we observed that individuals who had stopped heavy use of ecstasy for more than 1 year performed equally poor on the word recall test as recent heavy ecstasy users. The persistent memory problems in former ecstasy users may suggest irreversibility of ecstasy-induced serotonergic neurotoxic changes in brain regions involved in memory functions. In line with this, it has been shown in non-human primates that cortical serotonin terminal markers remain decreased up to 7 years after MDMA treatment, particularly prominent in the hippocampus <sup>18</sup>, although in humans hippocampal and cortical serotonin terminals seem to be reversible <sup>3,21</sup>. These studies have also shown that the density of serotonin terminal markers does not correlate with extent of memory loss. This is an intriguing observation, as discussed elsewhere <sup>3</sup>, and may indicate that anatomical recovery does not necessarily infer

functional recovery. However, future studies will have to investigate this point. We have previously published this part of the study<sup>3</sup>. However, in the present study the effects of ecstasy on other memory tests and other cognitive domains of that same population are presented, as well as possible confounding thereof by 5-HTTLPR. Few studies have investigated the long-term effects of ecstasy on cognitive function. Persistent impairments in former ecstasy users (abstinent for at least 6 months) in verbal recall performance and (visuo-spatial) working memory have been described compared to polydrug using controls<sup>52-54</sup>. Their performance on other cognitive tests was generally normal, like in our study. Thomasius *et al.* only observed reduced verbal recall performance in ex-users, and not in current heavy users<sup>5</sup>.

The present observations made in heavy users of ecstasy are generally consistent with previous reports suggesting that recreational ecstasy users display significant memory impairments, whereas performance on other cognitive tests is generally normal<sup>14,15,55-58</sup>. Impairments have been demonstrated in immediate and delayed verbal recall<sup>15,59-61</sup> and in verbal working memory<sup>62</sup>. Presently, differences in memory function between ecstasy users and controls were observed only using RAVLT. This may result from the fact that the RAVLT is known to be a very reliable test. Test re-test correlation scores (with an interval of 2 months) for RAVLT are higher than for the other memory tests administered in this study: 0.80 and 0.83 for RAVLT immediate and delayed recall, respectively<sup>63</sup>. For the other memory tests administered in this study the correlation coefficient varies from 0.60 (WMS) to 0.75 (Logical memory) (derived from WMS-III, 1997<sup>64</sup>).

Contrary to findings in previous studies in which ‘novice’ ecstasy users<sup>15,65</sup> demonstrated verbal memory deficits, we did not observe memory deficits in moderate users who had been exposed to less than 50 tablets in their life, 29 on average. Discrepancies between the previous studies may be attributed in part to the fact that subjects in our study abstained from psychoactive drugs for at least 3 weeks. Thus, acute or partial residual effects, or drug withdrawal, may have caused the memory disturbances noted in the study by Parrott *et al.*<sup>15</sup>. Alternatively, subjects in the previous mentioned studies may have used extremely high doses of ecstasy, causing brain serotonergic neurotoxicity despite the small number of separate drug exposures. One other study reported memory problems in moderate ecstasy users<sup>66</sup>. However, the moderate users had used on average 169 tablets (lifetime), as opposed to 29 tablets in the current study. In any case, it is well known from animal studies that higher dosages of ecstasy produce greater neurotoxic lesions<sup>17</sup>. In a study by Bolla and colleagues in which CSF 5-HIAA and memory function was assessed in abstinent ecstasy users, only individuals with more profound decrements in CSF 5-HIAA (presumably reflecting a greater extent of serotonin injury) had detectable difficulties with memory function<sup>59</sup>. In line with this, we previously reported that post-synaptic cortical 5-HT<sub>2A</sub> receptor availability was increased in ecstasy users

(presumably reflecting lower synaptic serotonin levels) and correlated positively with RAVLT-recall in ecstasy users<sup>67</sup>.

We presently investigated the potentially confounding influence of heritable effects on memory function and the use of ecstasy. In a small sample size we observed that the 5-HTTLPR genotype was not associated with memory function or ecstasy use. Although studies observed an important role of serotonin transporters in cognitive processes such as memory function<sup>24</sup>, we previously did not observe a correlation between memory function and cortical serotonin transporter densities obtained in a subset of the same subjects presented here<sup>3</sup>. Although there are studies suggesting that the *s* allele is associated with depression and anxiety-related personality traits<sup>26,27</sup>, other studies failed to find such an association<sup>68,69</sup>. Thus, the findings of the present study suggest that the observed memory deficits in ecstasy users do not result from a genetic predisposition to low serotonin transporter densities (the *s* allele), but probably result from the use of ecstasy itself, although pre-existing differences in memory performance cannot be ruled out. Furthermore, the use of ecstasy does not seem to result from pre-existing differences in serotonin transporter densities, since genotype distribution in ecstasy users was in good accordance with 5-HTTLPR genotype found in healthy European subjects<sup>26</sup>, and did not differ from the distribution found in control subjects. Furthermore, in the present study we were not able to replicate recent findings suggesting that ecstasy users carrying the *s* allele are at particular risk of developing serotonin related functional abnormalities<sup>33</sup>. However, because of our small sample size and that in the study of Roiser, more studies are needed with larger sample sizes to address this issue.

McCann and co-workers observed greater reductions in 5-HIAA in female than in male ecstasy users<sup>22</sup>. In line with this, we and others<sup>2,21</sup> observed greater reductions in serotonin transporter densities in female than in male ecstasy users, suggesting that females are more susceptible than males to the neurotoxic effects of ecstasy. Stronger anxiety effects in response to MDMA in females compared to males have been reported<sup>19</sup>. In addition, Verheyden *et al.* reported higher mid-week depression scores in female ecstasy users<sup>20</sup>. In contrast to this, we presently did not observe differences between males and females in the effects of ecstasy on memory function. Other studies have failed to investigate the effect of gender on memory function in ecstasy using subjects, or also did not observe an effect<sup>5,58,70</sup>, although Bolla and colleagues reported that females were less susceptible than males to ecstasy dose-related decrease in memory function<sup>59</sup>.

It is common for ecstasy users to consume cannabis, making it difficult to recruit ecstasy users who have not also used cannabis. Recent studies have pointed out the importance of taking cannabis consumption into account when studying ecstasy-related cognitive impairment<sup>71-74</sup>. However, the adverse effects of long-term cannabis use on cognitive skills appear to be short-term only. Pope *et al.* recently

showed that heavy cannabis users scored significantly below control subjects on a word recall list, which was detectable at least 7 days after heavy cannabis use, but not after 28 days<sup>75</sup>. Furthermore, animal studies have recently shown a protective effect of cannabinoid receptor agonists on MDMA-induced serotonin depletion, as well as on anxiety<sup>76</sup>. In the present study, three lines of evidence suggest that the deficits in the heavy recent and former ecstasy users discussed above were not related to cannabis consumption. The first is that if cannabis was responsible for the observed memory impairments then a significant covariance effect of cannabis on memory function in the MANOVA analysis might be expected, which was not the case. The second piece of evidence is that no association between extent of previous cannabis use and memory function was observed after controlling for potential confounders, as was observed for extent of previous ecstasy use. Previous studies have also failed to demonstrate an association in ecstasy users between extent of previous cannabis use and memory function<sup>61,65</sup>. Finally, the poor memory performance in heavy and former ecstasy using subjects is unlikely to be due to acute or partial residual effects of cannabis since all participants reported that they had abstained from use of cannabis or other psychoactive drugs for at least 3 weeks before the study, which was checked in the urine. Thus, although cannabis may have contributed to some extent to the poorer performance of heavy and former ecstasy users compared with ecstasy-naïve subjects, cannabis is unlikely to fully account for the present findings. We cannot exclude the possibility that the use of other drugs than ecstasy and cannabis (as discussed above) may have differed between groups and have contributed to the impairments observed here. We minimized the influence of other drugs than ecstasy and psychosocial factors by taking a control group from the same population of which the ecstasy users were recruited from. This differs conspicuously from most previous studies, where controls came from a university or general population.

Unfortunately, we were not able to assure abstinence from ecstasy, other than the past 3 days in the former ecstasy users. In future studies, hair-sample analysis may be useful to ascertain long periods of abstinence from ecstasy. In addition, follow-up studies in human subjects with known ecstasy-induced neurotoxicity need to be conducted to allow definite conclusions on reversibility of memory impairments in humans.

The observed memory impairments in heavy and former ecstasy users cannot readily be attributed to differences in verbal language skills, since the groups were all comparable with one and another on a measure of verbal IQ (DART). Likewise, it seems unlikely that they reflect generalized impairments of attention or concentration, since the groups did not differ on any of the tasks investigating these factors.

In summary, our data suggest dose-dependent decreases in memory function in ecstasy users, which may not be reversible since individuals who had stopped using ecstasy more than 1 year ago have impaired memory function, similar to recent ecstasy

users. In addition, our data provide no evidence for a role of 5-HTTLPR genotype in cognitive performance or ecstasy (ab)use, nor gender differences in susceptibility to ecstasy-induced memory impairment.

## ACKNOWLEDGEMENTS

The authors wish to thank Stefanie Jansen for carrying out the neuropsychological assessments. This study was partially supported by a grant from the Netherlands – Ministry of Health, Welfare and Sports (VWS) (GVM grant no. 43894).

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**PART**

**IV**

**Prospective studies in low-dose  
ecstasy users**

# CHAPTER 10

# **A Prospective Cohort Study on Sustained Effects of Low-Dose Ecstasy Use on the Brain in New Ecstasy Users**

Neuropsychopharmacology, in press; advance online publication 2006  
doi:10.1038/sj.npp.1301225

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# ABSTRACT

## Background

It is debated whether ecstasy use has neurotoxic effects on the human brain and what the effects are of a low dose of ecstasy use.

## Aim

We prospectively studied sustained effects (> 2 weeks abstinence) of a low dose of ecstasy on the brain in ecstasy-naive volunteers using a combination of advanced MR techniques and self-report questionnaires on psychopathology as part of the NeXT (Netherlands XTC Toxicity) study.

## Material and Methods

Outcomes of proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS), diffusion tensor imaging (DTI), perfusion-weighted imaging (PWI), and questionnaires on depression, impulsivity, and sensation seeking were compared in 30 subjects (12M, 21.8 ± 3.1 years) in two sessions before and after first ecstasy use (1.8 ± 1.3 tablets). Interval between baseline and follow-up was on average 8.1 ± 6.5 months and time between last ecstasy use and follow-up was 7.7 ± 4.4 weeks.

## Results

Using <sup>1</sup>H-MRS, no significant changes were observed in metabolite concentrations of *N*-acetylaspartate (NAA), choline (Cho), myo-inositol (mI) and creatine (Cr), nor in ratios of NAA, Cho, and mI relative to Cr. However, ecstasy use was followed by a sustained 0.9% increase in fractional anisotropy (FA) in frontoparietal white matter, a 3.4% decrease in apparent diffusion (ADC) in the thalamus and a sustained decrease in relative regional cerebral blood volume (rrCBV) in the thalamus (-6.2%), dorsolateral frontal cortex (-4.0%), and superior parietal cortex (-3.0%) (all significant at  $p < 0.05$ , paired *t*-tests). After correction for multiple comparisons, only the rrCBV decrease in the dorsolateral frontal cortex remained significant. We also observed increased impulsivity (+3.7% on the Barratt Impulsiveness Scale) and decreased depression (-28.0% on the Beck Depression Inventory) in novel ecstasy users, although effect sizes were limited and clinical relevance questionable.

## Conclusions

Because no indications were found for structural neuronal damage with the currently used techniques, our data do not support the concern that incidental ecstasy use leads to extensive axonal damage. However, sustained decreases in rrCBV and ADC values may indicate that even low ecstasy doses can induce prolonged vasoconstriction in some brain areas, although it is not known whether this effect is permanent. Additional studies are needed to replicate these findings.

## INTRODUCTION

There is increasing evidence that ecstasy (3,4-methylenedioxymethamphetamine, MDMA) is toxic to the human brain, especially to the serotonergic system<sup>1,2</sup>, although the validity of these findings is still highly debated<sup>3-5</sup>. Many human studies are littered with methodological problems, including inadequate sampling of subjects and controls, lack of drug use analysis, and lack of baseline data<sup>6,7</sup>. The latter argument leads to interpretative difficulties concerning causality between ecstasy use and potential toxicity, because it leaves open the possibility that differences between ecstasy users and controls were pre-existent, as discussed previously by others<sup>8-10</sup>. It may be possible that personality traits like impulsivity and sensation seeking, associated with substance misuse, are related to lower serotonergic function<sup>11,12</sup> or that results are biased by confounding variables such as polydrug use, gender, and lifestyle.

Few prospective studies were performed in which MDMA was administered to volunteers either with<sup>13-16</sup> or without prior experience with ecstasy use<sup>17-22</sup>. Most of these studies focused on acute and not on sustained or permanent effects of ecstasy. These studies have led to ongoing discussion on safety and ethics of administration of potentially neurotoxic drugs to healthy humans. Several authors objected to administering a potential neurotoxic drug to humans for the purpose of science<sup>23,24</sup>, while others supported these experiments<sup>25-27</sup>.

The discussion mainly persists, because it is assumed that heavy ecstasy use most probably causes adverse long-term effects<sup>28,29</sup>, while it is not known whether a low dose of ecstasy can cause lasting brain damage. Effects of a single dose of ecstasy in ecstasy-naïve humans were only described up to 24 hours after intake<sup>30</sup>, while persisting psychopathology after a single dose was only described in case reports<sup>31,32</sup>. In rats, neuronal damage was demonstrated in various brain areas following a single dose of MDMA<sup>33,34</sup>, including persistent effects on behaviour<sup>35</sup>. In addition, studies in primates showed serotonin depletion two weeks after administering a single (5 mg/kg)<sup>36</sup> or two oral doses (4.3 mg/kg)<sup>37</sup>. The validity of animal data for the human situation has been questioned, however, because MDMA is usually administered to animals in higher dosages than generally used by humans. Some authors do not support the suggestion that a single oral dose at 1.7 mg/kg is likely to produce neurotoxic effects in humans<sup>38-40</sup>. On the other hand, it has been advocated that these dosages in animals are equivalent to typical recreational dosages in humans according to the principles of interspecies scaling<sup>41,42</sup>.

It is important to know whether a low dose of ecstasy is neurotoxic for at least two reasons. First, recreational use of ecstasy is common among adolescents and young adults and many of them are 'experimenters' who take ecstasy incidentally and will not become heavy or regular users<sup>43</sup>. Determining that the incidental use of ecstasy could cause persisting neuronal damage would have major clinical and

social implications. Second, it is debated whether MDMA should become available for medical use, because MDMA may be useful as an adjunct in psychotherapy, or whether this would lead to neuronal damage<sup>44</sup>. This discussion is of interest, since pilots have been approved that will study therapeutic effects of MDMA on anxiety in patients with posttraumatic stress disorder<sup>45</sup> and in terminally-ill cancer patients<sup>46</sup>. When considering ecstasy as adjunct in psychotherapy, it is important that estimations of risk are available to decide whether potential risks outweigh potential benefits.

With advanced magnetic resonance (MR) techniques such as proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS), diffusion tensor imaging (DTI), and perfusion weighted imaging (PWI) it is possible to study various aspects of neuronal damage in the brain.

<sup>1</sup>H-MRS allows to study certain metabolites in the brain, such as *N*-acetylaspartate (NAA), choline-containing compounds (Cho), myo-inositol (mI), and (phospho)creatine (Cr). NAA is decreased in neuronal damage and impaired cognition<sup>47</sup>. Cho is increased in brain diseases that involve increased membrane breakdown, myelination or inflammation, and it is thought to reflect cellular density<sup>48</sup>. mI is a putative glial cell marker increased in diseases that involve gliosis<sup>49</sup>. Cr is often used as an internal reference<sup>50</sup>. Previous studies in heavy ecstasy users showed decreased NAA/Cr in the prefrontal cortex<sup>51</sup>, correlated to decreased memory function<sup>52</sup> and increased mI/Cr in the parietal white matter<sup>53</sup>. However, another study could not confirm lower NAA/Cr ratios in cortical brain regions, and observed only a tendency towards lower NAA/Cr ratios in the left hippocampus of ecstasy users<sup>54</sup>.

With DTI it is possible to measure diffusional motion of water molecules. In the brain, the motion is restricted in amplitude and direction by cellular structures such as axons. (Sub)acute processes that involve axonal injury and ischemia can lead to a decreased apparent diffusion coefficient (ADC) of water due to cytotoxic edema<sup>55</sup>. However, in chronic stage of axonal damage, ADC can be increased and fractional anisotropy (FA) of water can be decreased due to increased extracellular water content. It is difficult to determine the time course of change in ADC. For stroke, transition from decreasing to increasing ADC values seems to occur between 18 h and seven days after stroke onset<sup>56</sup>, while delayed cytotoxic edema with restricted ADCs was described up to 6 months, after carbon monoxide poisoning<sup>57,58</sup>, and heroin abuse<sup>59</sup>. Only one article was published with ADC measurements in heavy ecstasy users, and showed increased ADC values in the globus pallidus<sup>60</sup>.

Finally, PWI can map regional relative cerebral blood volume (rrCBV) using the dynamic susceptibility contrast (DSC) technique<sup>61,62</sup>. This is of interest because serotonin is involved in regulation of brain microcirculation<sup>63</sup>. Previous publications described cerebrovascular accidents, such as cerebral infarction and haemorrhage,<sup>64,65</sup> and cerebrovascular changes<sup>66-68</sup> in ecstasy users. It is expected that ecstasy use induces a (sub)acute increase of extracellular serotonin leading to vasoconstriction, whereas

the long-term effect of ecstasy use may be a decrease in extracellular serotonin and thus vasodilatation.

If a low dose of ecstasy is neurotoxic, it is important to know whether this has clinical consequences in terms of psychopathological parameters such as mood, impulsivity, and sensation seeking. Many previous studies reported increased levels of depression<sup>69</sup>, impulsivity<sup>70-76</sup>, and sensation/novelty seeking<sup>77-80</sup> in ecstasy users, although it is unclear whether these associations reflect a causal relationship, that is whether ecstasy use causes changes in mood, impulsivity, and sensation seeking or whether depression, impulsivity, and sensation seeking increase the probability of (heavy) ecstasy use (see also<sup>81</sup>).

The aim of the present study was to assess whether a low dose of ecstasy would be neurotoxic. Using a prospective naturalistic study design, parameters of neurotoxicity measured with <sup>1</sup>H-MRS, DTI and PWI and outcomes of psychopathological self-report inventories on depression, impulsivity, and sensation seeking were compared between a baseline session before first ecstasy use and a follow-up session after ecstasy use. Based on previous findings, we hypothesized that if a low dose of ecstasy has sustained effects on the brain MR-derived parameters and psychopathology would show relatively small changes between both sessions, i.e., a decrease (in the subacute stage shortly after ecstasy use) or increase (after a longer period of abstinence) in rrCBV and ADC, depending on the time since last ecstasy use; an increase in Cho (or Cho/Cr), mI (or mI/Cr), depression, impulsivity, and sensation seeking; and a decrease in FA and NAA (or NAA/Cr).

## METHODS AND MATERIALS

### Participants

The current study is part of the NeXT (Netherlands XTC Toxicity) study, which investigates causality, course, and clinical relevance of ecstasy neurotoxicity. A detailed description of the NeXT study and recruitment strategies can be found in a special design paper<sup>7</sup>. Between April 2002 and April 2004, 188 young adults (77 M, 111 F, age  $21.7 \pm 3.0$  years) were included in the study. They had never used ecstasy, but were selected on a relatively high probability to start using ecstasy in the near future. Subjects were recruited using a combination of targeted site sampling, advertisement through a website on the project, and snowball sampling referrals. Main inclusion criteria were intention to probably or certainly use ecstasy for the first time in near future and/or having friends who already used ecstasy. Exclusion criteria were: ecstasy use in the past (at baseline), age below 18 or above 35 years, severe physical or mental illness, use of psychotropic medications such as serotonin reuptake

inhibitors, pregnancy, use of intravenous drugs, and contraindications for MRI (e.g., claustrophobia, pacemaker). Subjects had to abstain from psychoactive substances for at least two weeks and from alcohol for at least one week before examinations. This was checked by urine drug screening (enzyme-multiplied immunoassay for amphetamines, ecstasy, opiates, cocaine, benzodiazepine, cannabis, and alcohol).

The study was approved by the local medical ethics committee. Subjects were informed about potential negative consequences of ecstasy use and all subjects signed informed consent. Subjects received an allowance for their participation (between €100 and €150 per session).

## Study procedure and measurements of confounders

At baseline all 188 subjects underwent MR imaging, including  $^1\text{H}$ -MRS, DTI, and PWI, and completed self-report questionnaires on depression, impulsivity, and sensation seeking. After baseline examination, subjects had to complete questionnaires (four in total) about their drug use at regular intervals over a period of approximately 18 months. For the present study, the first 31 incident ecstasy users were included in a first follow-up session, relatively soon after their first ecstasy use (after we received their first drug use questionnaire indicating use of ecstasy) and with a maximum cumulative ecstasy dose of 10 tablets. During the follow-up session,  $^1\text{H}$ -MRS, DTI, PWI, and self-report questionnaires on depression, impulsivity, and sensation seeking were repeated.

At both sessions, subjects had to complete questionnaires about potential confounders, such as demographic variables and education. Various aspects of lifetime ecstasy use (frequency of use, cumulative dose, and duration of use), and last year use of alcohol (units per week), tobacco (cigarettes per week), cannabis (number of joints last year), amphetamines (number of times used last year), and cocaine (number of times used last year) were assessed using substance-use questionnaires<sup>82</sup>. Verbal intelligence was estimated using The Dutch Adult Reading Test (DART), the Dutch version of the National Adult Reading Test<sup>83</sup>.

## Image acquisition

MR imaging was performed on a 1.5 T scanner (Signa Horizon, LX 9.0, General Electric Medical Systems, Milwaukee, WI, USA) using the standard head coil. The protocol included (1) an axial PD- and T2-weighted sequence (Echo Time ( $\text{TE}$ )<sub>1</sub> /  $\text{TE}$ <sub>2</sub> / Relaxation Time (TR) = 10/98/4000 ms, 12 slices of 5 mm, 1.5 mm slice distance, 23 cm field of view (FOV)); (2) three  $^1\text{H}$ -MRS scans with the single voxel point-resolved spectroscopy (PRESS) sequence (TE/TR = 35/1500 ms); (3) DTI: diffusion-weighted spin echo Echo Planar Imaging (EPI) (TE/TR = 90/8000 ms,

12 slices of 5 mm, 1.5 mm slice distance, 23 cm FOV;  $b = 0$  and  $1000 \text{ s/mm}^2$ ,  $128 \times 128$  matrix); (4) PWI: gradient echo EPI first-pass dynamic  $T_2^*$ -weighted contrast-enhanced imaging (TE/TR = 55/2000 ms, 12 slices of 5 mm, 1.5 mm slice distance, 23 cm FOV); and (5) a high resolution T1-weighted 3D scan using a Fast Spoiled Gradient Echo (FSPGR) sequence (TE/TR = 6/30 ms, voxel size  $1.0 \times 1.0 \times 1.4 \text{ mm}$ ). Throughout the study positioning of subjects in the scanner and positioning of the slices and voxels were performed by the same examiner and according to a protocol to keep positioning as reproducible as possible.

The voxel size for  $^1\text{H-MRS}$  was  $6.5 \text{ ml}$  ( $18 \times 18 \times 20 \text{ mm}^3$ ) and voxels were placed in the left centrum semiovale (frontoparietal white matter) and in mid-frontal and mid-occipital grey matter as in previous publications<sup>84,85</sup>. Shimming and water suppression were automatically performed by the scanner. Diffusion was measured in six non-collinear directions and in the six opposite directions. For each of these 12 directions ( $b = 1000 \text{ s/mm}^2$ ) and for a baseline measurement without diffusion weighting ( $b = 0 \text{ s/mm}^2$ ), two acquisitions were averaged. Perfusion images were obtained at 2-s intervals for 80 s. At 6 s after the start of image acquisition, a bolus ( $0.12 \text{ ml/kg}$ ) with gadobutrol  $1.0 \text{ mol/l}$  (Gadovist 1.0; Schering, Berlin, Germany) was injected, using a power-injector (Spectris MR injector; Medrad, Indianapolis, Pa) at a rate of  $5 \text{ ml/s}$  through a cannula inserted in the antecubital vein. The gadobutrol injection was followed by a  $15\text{-ml}$  saline flush ( $0.9\% \text{ NaCl}$ ).

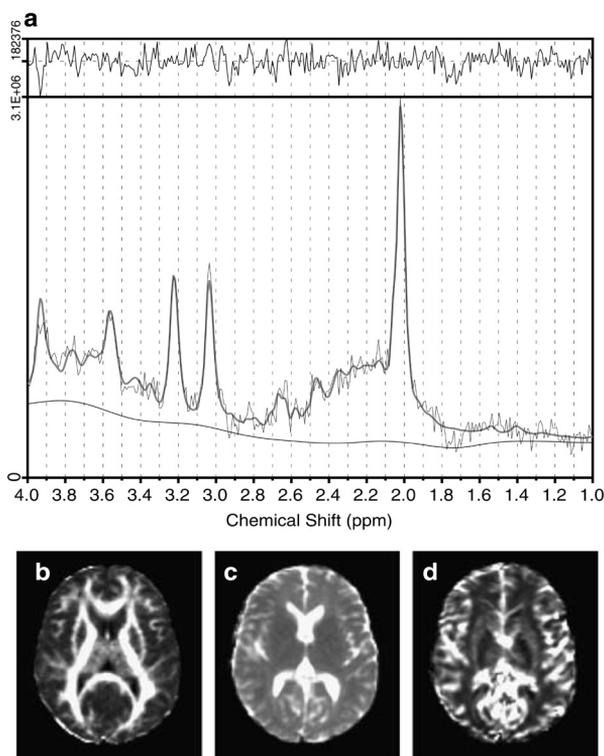
## Image Analysis

Spectra derived from  $^1\text{H-MRS}$  were analyzed using LCModel (Linear Combination of Model spectra)<sup>86</sup>. This is a user-independent analysis method that estimates absolute metabolite concentrations by fitting the *in vivo* spectra to a set of previously acquired *in vitro* spectra (the basis set). This procedure allows the absolute quantification of metabolite concentrations. Both absolute concentrations of NAA, Cho, mI, and Cr as well as the ratios of NAA to Cr, Cho to Cr, and mI to Cr were calculated with LCModel. Quality control of  $^1\text{H-MRS}$  included checking of line-width and the percent SD of the estimated concentrations after analyses by LCModel. Unsuppressed spectra with a waterpeak line-width of more than 6 Hz were excluded. Also  $\%SD > 20\%$  for NAA and  $\%SD > 50\%$  for Cho, mI and Cr were considered unreliable and were excluded<sup>87</sup>.

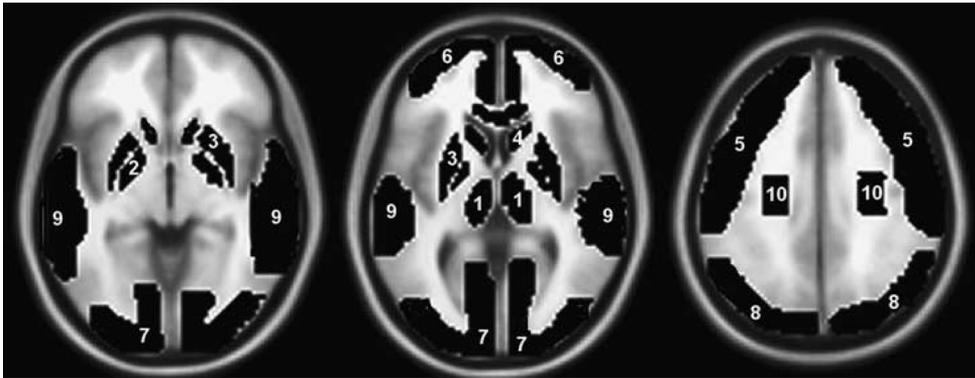
The DTI scans were corrected for the effects of residual eddy currents by matching the images acquired with opposite diffusion sensitizing gradients to each other with an affine transformation, and then correcting both images with the 'half' of that transformation<sup>88</sup>. From the resulting diffusion weighted images, ADC and FA maps were calculated as described elsewhere<sup>89</sup>. The AMC Postprocessing Package (APP,

<http://amcpostpack.sourceforge.net>) was used to calculate CBV maps from the PWI scans.

All image-derived parameters (FA, ADC, and CBV) were spatially normalized by registration to the Montreal Neurological Institute brain template (MNI152) in three steps. First, the scans corresponding to baseline measurements in the DTI and PWI sequences were individually matched to the T2-weighted images by 2D-non-rigid registration (program `align_warp`, Automated Image Registration Library, AIR<sup>90</sup>). Second, the T2 scans were rigidly registered to the T1-3D scans (program `flirt`, fMRIB Software Library, FSL<sup>91</sup>). Finally, the T1-3D scans were registered to the MNI152 brain template by a non-rigid transformation (`align_warp`). The transformations calculated to align the baseline measurements into T2, T2 into T1-3D, and T1-3D into MNI152 were applied to align the FA, ADC and CBV maps to the MNI152 brain (see Figure 1 for representative images of individual FA, ADC, and CBV images after transformation to the MNI152 brain template). All images were skull-stripped (program `bet`, FSL<sup>92</sup>). Segmentation of white and grey matter was performed using T1-3D and PD scans (program `fast`, FSL<sup>93</sup>). The scans were segmented into three classes of tissue (CSF, white, and grey matter), and the tissues of interest were isolated into separated binary maps (only white matter, only grey matter, and combined white and grey matter). The CBV maps were intensity-scaled to mean



**Figure 1:** Representative images of an individual (a) <sup>1</sup>H-MR spectrum after analysis by LCModel and representative (b) FA, (c) ADC, and (d) CBV images after transformation to the spatially normalized MNI brain template.



**Figure 2:** Region of interests used for analyses of DTI (measuring FA and ADC) and PWI (measuring rrCBV) drawn on the MRI brain template at three levels: (1) thalamus, (2) globus pallidus, (3) putamen, (4) caudate nucleus, (5) dorsolateral frontal cortex, (6) mid-frontal cortex, (7) occipital cortex, (8) superior parietal cortex, (9) temporal cortex, and (10) white matter of the centrum semiovale. Note that rrCBV was measured in all these ROIs and FA and ADC only in the white matter and basal ganglia ROIs.

individual CBV intensity of white matter derived from the segmentation procedure to generate relative CBV (rCBV) maps.

Regions of interest (ROIs) were drawn on the MNI152 brain template in the thalamus, putamen, globus pallidus, head of the caudate nucleus, centrum semiovale (frontoparietal white matter), and dorsolateral frontal, mid-frontal, occipital, superior parietal, and temporal cortex (see Figure 2). For the cortical ROIs, only voxels within the grey matter mask were included and for the ROIs of the basal ganglia only voxels within the combined white and grey matter mask were included (CSF voxels were excluded). Selection of ROIs was based on findings of previous studies, which indicated that ecstasy-induced abnormalities are most prominent in basal ganglia and cortical areas; ecstasy-induced abnormalities in white matter were rarely reported and thus not expected. As cortical grey matter has very low anisotropy, it is very difficult to get reliable FA and ADC measurements in cortical areas. For this reason only white matter and basal ganglia ROIs were taken into account in the measurements of FA and ADC. Within the ROIs, individual mean values of FA, ADC, and regional relative CBV (rrCBV) were calculated. Values of FA, ADC and rrCBV from ROIs in left and right hemispheres were averaged.

For each individual scan, all steps in the post-processing and analysis were visually inspected to check the quality of image registration and segmentation.

## Psychopathological assessments

Current depressive symptoms were assessed using the Beck Depression Inventory (BDI)<sup>94</sup>, a 21-item self-report inventory that measures characteristic attitudes and

symptoms of depression in the week prior to assessment. Impulsivity was assessed using the Dutch version of the Barratt Impulsiveness Scale (BIS-11), a reliable measure of impulsiveness<sup>95</sup>. The Dutch BIS-11 contains 31 self-reported items. The Spannings Behoefte Lijst (SBL), a Dutch adaptation of the Sensation Seeking Scale<sup>96</sup> with 51 items, was used to measure sensation seeking as it has proven to be a reliable measure for research populations<sup>97,98</sup>.

## Statistical analyses

All substance-use variables were log-transformed because they were not normally distributed. First, paired *t*-tests, uncorrected for multiple comparisons, were used to assess whether parameters of substance use, imaging, and self-report questionnaires had changed between baseline session before ecstasy use and follow-up session after ecstasy use.

Second, paired *t*-tests were repeated for imaging and psychopathology parameters excluding volunteers with increased cocaine use between both sessions ( $N = 4$ , leaving  $N = 26$  for the second analysis), because paired analysis of substance use showed an increased frequency of cocaine use between baseline and follow-up sessions.

Third, previous studies showed that effects of ecstasy might be dose-dependent<sup>99,100</sup> and that females are more vulnerable for the effects of ecstasy than males<sup>101,102</sup>. Other studies suggested a relationship between brain perfusion and time since last ecstasy use and between ADC and time since onset of neuronal damage<sup>103,104</sup>. Therefore, we performed separate multiple linear regression analyses with follow-up measures of imaging and self-reported psychopathology as dependent variable and gender, cumulative dose of ecstasy, period of abstinence (weeks since last ecstasy tablet) and change in cocaine use (because this was the only significantly increased drug-use parameter) as independent variable and baseline measures of imaging and self-reported psychopathology as covariates.

Finally, Pearson correlations were calculated between statistically significant changes in MR outcomes and significant changes in outcomes of the psychopathology questionnaires.

The chance of a type I error ( $\alpha$ ) was set at 0.05 for all analyses. In addition, Bonferroni *post hoc* corrections were performed for the analyses of the imaging parameters, adjusting the  $\alpha$ -level for multiple comparisons. The adjusted  $\alpha$ -level was set at 0.006 for <sup>1</sup>H-MRS outcomes (9 comparisons), at 0.010 for FA and ADC (5 comparisons each), and at 0.005 for rrCBV (10 comparisons).

All statistical analyses were performed using SPSS version 11.5; SPSS Inc., Chicago, IL, USA). Mean values reported in the result section are followed by their standard deviations (mean  $\pm$  SD). In the tables mean differences between the paired

measurements are reported with their 95% confidence intervals (95% CI) and in the text the % difference and the two-tailed significance level ( $p$ -values) are reported.

## RESULTS

### Characteristics of the sample and substance use

Of the 188 ecstasy-naïve subjects at baseline, 31 subjects were included in the first follow-up session relatively soon after their first ecstasy use (12 M, 19 F, age  $21.7 \pm 3.1$  years). One female was excluded because of a positive urine test on cocaine, leaving 30 volunteers for analysis with a mean age of 21.8 years. Characteristics of the sample and their substance use are described in Table 1. The interval between the baseline and follow-up sessions was on average  $8.1 \pm 6.5$  months (range: 0.9 - 29.5 months). At this first follow-up session incident ecstasy users had used a mean of  $1.8 \pm 1.3$  ecstasy

**Table 1:** Characteristics of demographics, use of ecstasy and other substances, and psychopathological assessments ( $N = 30$ ). Values expressed as mean  $\pm$  SD's. Results expressed in mean of paired differences (95% CI).

	Baseline before ecstasy use	Follow-up after ecstasy use	Mean of paired differences (95% CI) <sup>a</sup>
Gender	12 M, 18 F		na
Age	$21.8 \pm 3.1$	$22.5 \pm 3.2$	$0.67 (0.47; 0.87)^*$
Years of education	$14.2 \pm 2.8$	$14.8 \pm 2.9$	$0.57 (0.26; 0.87)^*$
DART-IQ	$104.6 \pm 8.5$		NA
<b>Ecstasy</b>			
Cumulative dose (tablets)	NA	$1.8 \pm 1.3$	NA
Time since first tablet (weeks)	NA	$9.8 \pm 4.5$	NA
Time since last tablet (weeks)	NA	$7.7 \pm 4.4$	NA
<b>Other substances</b>			
Alcohol (units/week)	$9.3 \pm 7.3$	$8.7 \pm 7.5$	$-0.10 (-0.27; 0.07)$
Tobacco (cig/week)	$23.4 \pm 39.0$	$17.4 \pm 30.2$	$-0.07 (-0.51; 0.36)$
Cannabis (joints in last year)	$36.2 \pm 52.0$	$38.4 \pm 52.6$	$-0.03 (-0.34; 0.28)$
Amphetamine (number of times used last year)	$0.2 \pm 1.1$	$0.2 \pm 1.1$	NA
Cocaine (number of times used last year)	$0.6 \pm 1.8$	$1.4 \pm 2.6$	$0.34 (0.01; 0.67)^*$
<b>Psychopathological assessments</b>			
Beck Depression Inventory	$4.8 \pm 4.0$	$3.4 \pm 3.4$	$-1.37 (-2.63; -0.10)^*$
Barratt Impulsiveness Scale	$67.5 \pm 6.8$	$70.0 \pm 7.8$	$2.50 (0.79; 4.21)^*$
SBL - Sensation Seeking Scale	$13.7 \pm 1.3$	$13.9 \pm 1.2$	$0.23 (-0.09; 0.55)$

<sup>a</sup> paired  $t$ -test baseline vs follow-up, substance use log-transformed

\* statistical significant difference between baseline and follow-up

tablets (range: 0.5 – 6; median 1.4 tablets). The majority had used ecstasy only once ( $N = 18$ ; 60%). Six subjects (20%) had used more than one ecstasy tablet at the same occasion with a maximum of two tablets per occasion. The interval between the last ecstasy use and follow-up measurements was  $7.7 \pm 4.4$  weeks.

Table 1 shows that besides the use of ecstasy, there was a significant increase in cocaine use between sessions ( $p = 0.043$ ), while there was no change in use of other substances.

## **<sup>1</sup>H-MRS, DTI, and PWI**

The maximum line-width of the unsuppressed spectra was 6 Hz and maximum %SD of the estimated metabolite concentrations was 20% for NAA and Cho and 25% for mI, and therefore, all spectra could be included. However, due to technical problems with the scanner it was not possible to perform <sup>1</sup>H-MRS in two subjects at the follow-up session.

Anatomical images (T1 3D scans and T2-weighted scans) were read by a neuroradiologist for atrophy or white matter lesions, and no significant abnormalities were detected. However, one subject had enlarged lateral ventricles and visual inspection showed this hampered matching to the standard brain, and therefore the measurements of FA, ADC, and rrCBV of this subject were not included. Therefore, we report comparisons between baseline and follow-up measurements of FA, ADC, and rrCBV in 29 subjects and of <sup>1</sup>H-MRS in 28 subjects.

Table 2 shows results of all measurements and Figure 3 illustrates the statistically significant findings. There were no significant changes in absolute concentrations of NAA, Cho, mI and Cr and in ratios of NAA, Cho, or mI relative to Cr in any of the three voxels after ecstasy use. With DTI, we observed a small but significant increase of 0.9% in FA of the white matter of the centrum semiovale ( $p = 0.027$ ) and a significant decrease of 3.4% in ADC of the thalamus ( $p = 0.015$ ) after ecstasy use. With PWI we found significant decreases in rrCBV in the thalamus (-6.2%,  $p = 0.010$ ), dorsolateral frontal grey matter (-4.0%,  $p = 0.001$ ), and superior parietal grey matter (-3.0%,  $p = 0.029$ ) (Figure 3). When adjusted for multiple comparisons using the Bonferroni correction, only the decreased rrCBV value in the dorsolateral frontal grey matter remained statistically significant.

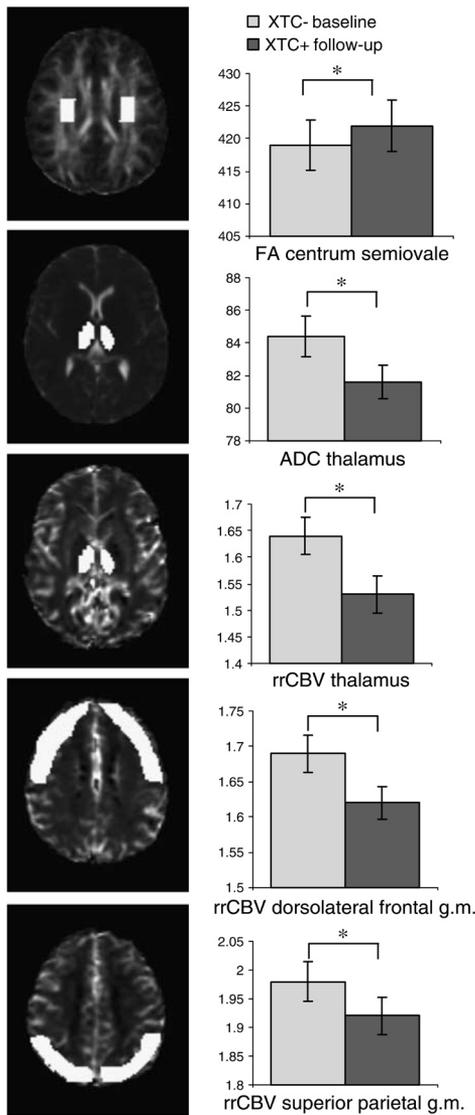
Similar to the first set of analyses, the second set of analyses, excluding subjects with increased cocaine use between both sessions, showed no significant changes in metabolite concentrations and ratios. Similar to the first analysis, it showed a significant decrease of ADC in the thalamus (-3.9%,  $p = 0.011$ ) and of rrCBV in the thalamus (-6.7%,  $p = 0.010$ ), dorsolateral frontal cortex (-4.2%,  $p = 0.002$ ) and superior parietal grey matter (-3.4%,  $p = 0.026$ ). However, the increase in FA in the white matter of the centrum semiovale was not significant anymore ( $p = 0.085$ ) and the second analysis

**Table 2:** MRI parameters before and after first ecstasy use. Values expressed as mean  $\pm$  SD's. Results expressed in mean of paired differences (95% CI).

MR technique	Parameter	Region of interest	Baseline before ecstasy use	Follow-up after ecstasy use	Mean of paired differences (95% CI)	
<sup>1</sup> H-MRS (N=28) absolute	NAA	mid-frontal grey matter	16.21 $\pm$ 2.33	15.8 $\pm$ 1.83	-0.41 (-1.55; 0.72)	
		mid-occipital grey matter	17.13 $\pm$ 1.81	17.44 $\pm$ 1.88	0.32 (-0.56; 1.20)	
		left centrum semiovale	13.65 $\pm$ 1.41	13.41 $\pm$ 1.56	-0.24 (-1.02; 0.55)	
	Cho	mid-frontal grey matter	3.10 $\pm$ 0.55	3.23 $\pm$ 0.57	0.13 (-0.14; 0.40)	
		mid-occipital grey matter	1.72 $\pm$ 0.25	1.75 $\pm$ 0.22	0.03 (-0.06; 0.12)	
		left centrum semiovale	2.98 $\pm$ 0.40	3.03 $\pm$ 0.52	0.05 (-0.08; 0.18)	
	mI	mid-frontal grey matter	11.93 $\pm$ 1.93	11.41 $\pm$ 1.79	-0.52 (-1.38; 0.34)	
		mid-occipital grey matter	9.26 $\pm$ 1.06	9.43 $\pm$ 0.95	0.18 (-0.41; 0.76)	
		left centrum semiovale	7.37 $\pm$ 0.98	7.80 $\pm$ 0.99	0.44 (-0.05; 0.92)	
	Cr	mid-frontal grey matter	13.42 $\pm$ 1.93	12.84 $\pm$ 1.78	-0.58 (-1.51; 0.34)	
		mid-occipital grey matter	11.59 $\pm$ 0.77	11.49 $\pm$ 0.72	-0.10 (-0.51; 0.31)	
		left centrum semiovale	9.02 $\pm$ 1.05	9.13 $\pm$ 1.09	0.11 (-0.36; 0.58)	
<sup>1</sup> H-MRS (N=28) ratio's	NAA/Cr	mid-frontal grey matter	1.23 $\pm$ 0.26	1.25 $\pm$ 0.20	0.02 (-0.11; 0.15)	
		mid-occipital grey matter	1.49 $\pm$ 0.18	1.53 $\pm$ 0.17	0.04 (-0.06; 0.13)	
		left centrum semiovale	1.52 $\pm$ 0.20	1.48 $\pm$ 0.29	-0.03 (-0.17; 0.10)	
	Cho/Cr	mid-frontal grey matter	0.23 $\pm$ 0.04	0.25 $\pm$ 0.05	0.02 (-0.00; 0.04)	
		mid-occipital grey matter	0.15 $\pm$ 0.02	0.15 $\pm$ 0.02	0.00 (-0.00; 0.01)	
		left centrum semiovale	0.33 $\pm$ 0.04	0.33 $\pm$ 0.05	-0.00 (-0.02; 0.02)	
	mI/Cr	mid-frontal grey matter	0.90 $\pm$ 0.14	0.90 $\pm$ 0.15	0.00 (-0.07; 0.08)	
		mid-occipital grey matter	0.80 $\pm$ 0.10	0.83 $\pm$ 0.10	0.02 (-0.03; 0.07)	
		left centrum semiovale	0.83 $\pm$ 0.13	0.86 $\pm$ 0.16	0.04 (-0.02; 0.10)	
	DTI (N=29)	FA (x1000)	thalamus	277 $\pm$ 18	278 $\pm$ 17	1.9 (-4.0; 7.7)
			globus pallidus	303 $\pm$ 59	295 $\pm$ 55	-8.0 (-28.6; 12.6)
			putamen	212 $\pm$ 30	212 $\pm$ 30	-0.2 (-10.4; 10.1)
caudate nucleus			176 $\pm$ 39	176 $\pm$ 29	-0.3 (-11.2; 10.7)	
centrum semiovale			<b>419 <math>\pm</math> 20</b>	<b>422 <math>\pm</math> 19</b>	<b>3.6 (0.4; 6.8)*</b>	
ADC 10-5 mm <sup>2</sup> /sec		thalamus	<b>84.4 <math>\pm</math> 6.6</b>	<b>81.6 <math>\pm</math> 5.3</b>	<b>-2.8 (-5.1; -0.6)*</b>	
		globus pallidus	72.4 $\pm$ 4.2	72.3 $\pm$ 2.9	-0.1 (-1.4; 1.3)	
		putamen	70.8 $\pm$ 1.9	70.8 $\pm$ 1.5	0.1 (-0.7; 0.8)	
		caudate nucleus	88.4 $\pm$ 11.8	86.0 $\pm$ 8.5	-2.4 (-5.3; 0.5)	
		centrum semiovale	70.0 $\pm$ 1.8	70.1 $\pm$ 2.0	0.1 (-0.4; 0.5)	
PWI (N=29)		rrCBV	thalamus	<b>1.64 <math>\pm</math> 0.18</b>	<b>1.53 <math>\pm</math> 0.16</b>	<b>-0.10 (-0.18; -0.03)*</b>
			globus pallidus	1.00 $\pm$ 0.14	1.06 $\pm$ 0.16	0.07 (-0.01; 0.14)
	putamen		1.36 $\pm$ 0.13	1.32 $\pm$ 0.12	-0.04 (-0.09; 0.01)	
	caudate nucleus		1.28 $\pm$ 0.12	1.25 $\pm$ 0.12	-0.04 (-0.08; 0.01)	
	dorsolateral frontal grey matter		<b>1.69 <math>\pm</math> 0.14</b>	<b>1.62 <math>\pm</math> 0.12</b>	<b>-0.07 (-0.11; -0.03)*,†</b>	
	mid-frontal grey matter		1.68 $\pm$ 0.17	1.65 $\pm$ 0.14	-0.02 (-0.07; 0.02)	
	occipital grey matter		2.16 $\pm$ 0.22	2.11 $\pm$ 0.20	-0.04 (-0.11; 0.03)	
	superior parietal grey matter		<b>1.98 <math>\pm</math> 0.17</b>	<b>1.92 <math>\pm</math> 0.16</b>	<b>-0.06 (-0.11; -0.01)*</b>	
	temporal grey matter		2.02 $\pm$ 0.21	1.98 $\pm$ 0.22	-0.04 (-0.11; 0.03)	
	centrum semiovale		0.76 $\pm$ 0.05	0.78 $\pm$ 0.07	0.01 (-0.01; 0.03)	

\* statistical significant difference between baseline and follow-up (paired *t*-test, uncorrected for multiple comparisons)

† statistical significant difference between baseline and follow-up (paired *t*-test, corrected for multiple comparisons)



**Figure 3:** On the left, FA, ADC and rCBV maps with brain regions, that significantly differed between baseline and follow-up (uncorrected for multiple comparisons), marked in white. On the right, columns reflect corresponding FA values in the centrum semiovale, ADC values in the thalamus, and rrCBV values in thalamus, dorsolateral frontal grey matter and superior parietal grey matter at baseline before (XTC-) and at follow-up after ecstasy use (XTC+). Results represent mean  $\pm$  SEM; \* =  $p < 0.05$ . Only significant results are shown, for complete results of all analyses see Table 2. Note that the vertical axis does not start at zero.

showed an additional significant decrease of rrCBV in the putamen (-3.8%,  $p = 0.047$ ). After correction for multiple comparisons, only the rrCBV value in the dorsolateral frontal grey matter remained statistically significant.

The linear regression analyses showed a significant effect of gender on Cho/Cr in the occipital grey matter ( $B = -0.02$ ,  $p = 0.038$ ), on Cho/Cr ( $B = -0.04$ ,  $p = 0.032$ ), and on rrCBV in the temporal grey matter ( $B = -0.17$ ,  $p = 0.021$ ). This means that females (assigned '2' in the analysis) showed a significant larger decrease in Cho/Cr and rrCBV than males (assigned '1' in the analysis). The total amount of ecstasy tablets had only a significant positive effect on FA in white matter ( $B = 3.35$ ,  $p = 0.009$ ) and the time since last ecstasy use had no significant effect on any of the outcome measures at the follow-up session. Increase in cocaine use was significantly related to an increase in mI and mI/Cr in the occipital grey matter ( $B = 0.23$ ,  $p = 0.033$  and  $B = 0.02$ ,  $p = 0.019$ , respectively). Of these regression analyses only the positive effect of the total amount of ecstasy tablets on FA in white matter remained significant after correction for multiple comparisons.

## Psychopathological assessments

Results of the self-report questionnaires on depression, impulsivity, and sensation seeking at baseline and

follow-up sessions are shown in Table 1. After ecstasy use (at the follow-up session), subjects scored significantly lower on symptoms of depression (-28.0%,  $p = 0.035$ ) and significantly higher on signs of impulsivity (3.7%,  $p = 0.006$ ). No changes were observed in sensation seeking. Similar to the first analyses, the second analyses, excluding subjects with increased cocaine use between both sessions, showed no significant changes in sensation seeking, significant lower symptoms of depression (-29.8%,  $p = 0.045$ ), and significantly higher signs of impulsivity (3.4%,  $p = 0.013$ ).

The linear regression analyses showed a significant positive effect of increased cocaine use on sensation seeking ( $B = 0.15$ ,  $p = 0.026$ ). There were no significant correlations between increased depression and impulsivity scores and significant changes in MR outcomes.

## DISCUSSION

To our knowledge, this is the first imaging study that prospectively examined sustained effects of a low dose of ecstasy on the human brain. Given the existing data on potential neurotoxicity, it is highly controversial to give ecstasy to ecstasy-naïve individuals in a controlled experiment<sup>105-108</sup>. Therefore, we used a naturalistic design in which young adults with a relatively high probability for first time ecstasy use were included in a follow-up study. Only a few subjects incidentally used amphetamines and cocaine, and the use of alcohol, tobacco, and cannabis before the two sessions was very similar.

<sup>1</sup>H-MRS and FA, parameters of structural elements of the brain, did not show indications of neuronal damage (i.e., no decrease in NAA, NAA/Cr, FA, and no increase in Cho, Cho/Cr, mI, mI/Cr) after the first use of a low dose of ecstasy. This is not unexpected, because previous observations showed that neurotoxic effects of ecstasy are probably dose-related<sup>109-111</sup>. Previous <sup>1</sup>H-MRS studies showed decreased NAA/Cr ratios in ecstasy users with an average cumulated dose of more than 700 tablets<sup>112,113</sup>, while others found no decreased NAA/Cr ratios in subjects with more moderate lifetime doses<sup>114,115</sup>. Therefore, these effects probably only appear after cumulative heavy use. On the other hand, we observed a small but significant decrease of 3.5% (Cohen's  $d = -0.47$ ) in ADC in the thalamus after first ecstasy use<sup>116</sup>. We can speculate that this might be related to ecstasy-induced cytotoxic edema, as observed in other neurotoxic substances<sup>117,118</sup>, although it also could be related to protracted vasoconstriction, since we also observed a decreased rrCBV in the thalamus (Pearson correlation between these findings is 0.65,  $p < 0.001$ ). With DTI we also encountered the unexpected finding of increased FA in the centrum semiovale related to the total amount of ecstasy tablets, although this 0.9% increase was very small (Cohen's  $d = 0.15$ ).

Functional parameters were measured with PWI and self-report questionnaires. As previously observed<sup>119</sup> we found an increase in rrCBV in the globus pallidus, although this effect was not significant ( $p = 0.09$ ). In addition, we found significant small to moderate decreases in rrCBV in dorsolateral frontal cortex, superior parietal cortex, and thalamus (Cohen's  $d = 0.36-0.65$ ). Decreases in cerebral blood flow (CBF), mainly in the caudate nucleus, and superior parietal and right dorsolateral frontal cortices, were previously observed after only two doses of MDMA<sup>120</sup>. Because this happened within 3 weeks after MDMA administering and because microcirculation in these areas has a strong relationship with serotonergic terminals<sup>121</sup>, the authors hypothesized that the decreased CBF was caused by sub-acute vasoconstriction due to MDMA-mediated serotonergic effects. The same study reported an increased CBF two to three months after MDMA intake (although only studied in two subjects) and no differences were found in CBF between controls and abstinent ecstasy users with a mean abstinence period of 6.6 months. They speculated that these findings might reflect depletion of serotonin after a longer period of abstinence and normalization of brain perfusion, respectively. Based on these results, they suggested a relationship between brain perfusion and abstinence period. Previous findings of higher rrCBVs in ecstasy users with an average period of abstinence of 14.6 weeks (globus pallidus)<sup>122</sup> and higher rrCBVs in former ecstasy users (globus pallidus and thalamus) than in recent ecstasy users and controls<sup>123</sup> could be in line with this hypothesis, although study populations were small. The latter study also showed low rrCBV values in combination with lower cortical 5-HT<sub>2</sub> receptor densities, suggesting down-regulation of 5-HT<sub>2</sub> receptors, in ecstasy users with a mean abstinence period of 7 weeks, and they hypothesized this was caused by excessive ecstasy-induced serotonin release. The currently observed decreased rrCBV values in subjects with a mean period of abstinence of 7.7 weeks might therefore also be related to a, probably transient, ecstasy-induced down-regulation of 5-HT<sub>2</sub> receptors, which play an important role in the regulation of brain microcirculation<sup>124,125</sup>. On the other hand, we did not find significant correlations between rrCBV and the time since last ecstasy tablet (abstinence interval). Another speculation is that decreased rrCBV values might reflect decreased brain function, because a single ecstasy dose was shown to cause degenerating neurons in parietal cortex and thalamus of rats<sup>126</sup>. In line with this, deficits in brain perfusion were reported in polydrug<sup>127</sup> and methamphetamine abusers<sup>128,129</sup>.

Outcomes of the self-report questionnaires after first ecstasy use showed increased impulsivity, as previously observed in other studies<sup>130</sup>. However, the magnitude of the effect is limited (3.7 % increase; Cohen's  $d = 0.34$ ) and the clinical relevance is therefore questionable. Subjects also reported lower levels of depression after ecstasy use than before, an unexpected finding that might be related to a euphoric feeling about the first ecstasy experience<sup>131</sup>. Also here, the clinical relevance of the reduction from 4.8 to 3.4 is questionable because the effect size is rather limited (Cohen's  $d = -$

0.38) and because BDI scores between 0 and 9 are considered to be within the normal range. Moreover, it should be noted that the findings were not reproduced after a longer follow-up period in a larger sample of the same baseline population<sup>81</sup>.

Although this study and some other studies showed that adverse effects of a low ecstasy dose are limited<sup>132,133</sup>, there are various factors (e.g., poor metabolism, hypertension, young age, simultaneous use of other substances, environmental conditions) that might contribute to individual or situational vulnerability for acute adverse effects and long-term neurotoxicity of ecstasy<sup>134-137</sup>. Therefore, it is not possible to state that incidental use of ecstasy is completely safe. For example, neurocognitive data from a larger sample of the current study population suggest that even low dose ecstasy use is associated with small but significant decreases in verbal memory relative to non-users<sup>138</sup>.

As we used multiple techniques as indicators for ecstasy-induced brain damage and multiple regions of interests, there is an increased probability of type I errors (false positive results). Therefore, additional *post-hoc* Bonferroni corrections on all imaging analyses were performed. The results showed that most of the significant findings did not remain significant after Bonferroni correction. On the other hand, the Bonferroni correction may be too conservative especially because *a priori* we expected small effects as we studied early indicators of potential brain damage in subjects with only *low* cumulative doses of ecstasy use. Moreover, all imaging techniques and ROIs were chosen based on *a priori* hypothesis. Therefore, it is likely that the Bonferroni-correction induces type II errors (false negative findings). The risk of such corrections was previously discussed by Rothman (1990) who showed that they can obscure possibly important findings<sup>139</sup>. As a result of its social impact, additional research is needed to establish whether the current uncorrected significant findings can be replicated.

A limitation of the present study is the uncertainty about variances in dosage and purity of ecstasy tablets, although pill-testing confirms that in the Netherlands more than 95% of the tablets sold as ecstasy contain MDMA as the only (91.2%) or major (4.2%) component<sup>43,140,140</sup>. The MDMA-related psychoactive substances 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA) or 3,4-methylenedioxyamphetamine (MDA) are major components in 1.5 % of the ecstasy tablets and only 1% of the ecstasy tablets contain amphetamine. The mean concentration of MDMA in an ecstasy tablet was 78 mg in 2003 in the Netherlands, but there is an increase in tablets with a dose of more than 140 mg MDMA<sup>43</sup>. Also the environmental circumstances under which ecstasy was taken and the simultaneous use of other substances was heterogeneous. As a result of these changing circumstances, it is possible that the observed changes in FA, ADC, rrCBV, depression and impulsivity are not related to ecstasy use, but to other time- or ecstasy-related variables. Confounding by the use of other substances, such as alcohol, nicotine, cannabis, amphetamines, and cocaine, cannot be totally

excluded, although alcohol, nicotine, cannabis, and amphetamine use did not change between sessions and most effects remained significant after exclusion of subjects with increased use of cocaine between sessions.

Another limitation is that we did not include a control group. Therefore, we cannot be completely sure about the reproducibility of our measurements. Other studies suggest that reproducibility of  $^1\text{H-MRS}$  <sup>141</sup>,  $\text{DTI}$  <sup>142,143</sup> and  $\text{PWI}$  <sup>144</sup> is good, although this might depend on the scanner, the scan protocol, and post-processing procedures. ROIs were drawn in the spatially normalized MNI brain template, which may have introduced additional variance due to inherent variations in mapping of individual brains to the MNI brain. On the other hand, compared to drawing ROIs for each individual subject, the current procedure is user-independent and reproducible, because the same procedure is performed for all subjects exactly in the same way. As few studies used  $^1\text{H-MRS}$ ,  $\text{DTI}$ , and  $\text{PWI}$  to study neuronal damage in ecstasy users, little is known about the sensitivity and specificity of these techniques to detect ecstasy-induced neuronal damage. Therefore, additional studies are needed, both in animals and in heavy human ecstasy users. As expected neuronal damage after a low dose of ecstasy is relatively small, the statistical power of this study could have been insufficient for  $^1\text{H-MRS}$  and  $\text{DTI}$  to detect changes.  $\text{DTI}$  is particularly suitable for detection of white matter lesions, while ecstasy-related neuronal damage is especially expected in basal ganglia and cerebral cortex. As these areas have low FA, the sensitivity of this parameter to detect axonal dysfunction in basal ganglia might be limited. On the other hand,  $^1\text{H-MRS}$ ,  $\text{DTI}$ , and  $\text{PWI}$  have been shown to be sensitive tools in various neuropsychiatric disorders. For example,  $^1\text{H-MRS}$  showed to be sensitive to detect changes in patients with schizophrenia, affective disorders, autism, and depression <sup>145,146</sup> and substance users <sup>147-149</sup>.  $\text{DTI}$  showed to be sensitive in detection of early diffuse axonal injury after traumatic brain injury <sup>150</sup> and various neuropsychiatric disorders <sup>151</sup>.  $\text{PWI}$  showed to be sensitive in detection of  $\text{rrCBV}$  deficits in early Alzheimer's disease <sup>152,153</sup> and in other neuropsychiatric diseases <sup>154</sup>.

In conclusion, with the currently used techniques we found no indications for structural neuronal damage after a low dose of ecstasy use in first time ecstasy users. Therefore, these data do not support the concern that incidental ecstasy use leads to serious axonal loss, although more studies are needed to assess the sensitivity of the currently used MR techniques to detect small ecstasy-induced neuronal changes. However, our findings of decreased  $\text{rrCBV}$  and  $\text{ADC}$  may indicate that even a low dose of ecstasy can induce sustained vasoconstriction in some brain areas, although we do not know whether these findings are permanent. Therefore, and because there may be various personal and environmental factors that play a role in the occurrence of acute and long-term effects of ecstasy, it is impossible to state, based on this study, that incidental use of ecstasy is totally safe for the brain.

## ACKNOWLEDGEMENTS

The NeXT study was supported by a grant of The Netherlands Organization for Health Research and Development as part of their Addiction Program (ZonMw 310-00-036). Sílvia D. Olabarriaga participates in the Virtual Laboratory for e-Science project, which is supported by a BSIK grant from the Dutch Ministry of Education, Culture and Science (OC&W) and is part of the ICT innovation program of the Ministry of Economic Affairs (EZ). Questionnaires on drug use were obtained by courtesy of the Addiction Research Institute of the University of Utrecht. We thank Professor M. Moseley (Lucas MRS Center, Stanford University) for support in the implementation of the DTI protocol. We thank Hylke Vervaeke for recruiting volunteers; Sarah Dijkink for assistance with data collection and Jeroen Snel for assistance with post-processing of DTI and PWI scans.

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# CHAPTER 11

# **Sustained Effects of Ecstasy on the Human Brain: a Prospective Neuroimaging Study in Novel Users**

Submitted

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# ABSTRACT

## Background

Previous studies suggest toxic effects of recreational ecstasy use to the serotonin system of the brain, but it cannot be excluded that observed differences between users and non-users are the cause rather than the consequence of ecstasy use.

## Aim

To prospectively assess sustained effects of ecstasy on the brain in abstinent novel ecstasy users using a combination of advanced neuroimaging techniques.

## Material and methods

Brain imaging parameters of neurotoxicity were assessed before and after a first period of ecstasy use and compared to those in persistent ecstasy-naïves using a prospective design, as part of the Netherlands XTC Toxicity (NeXT) study. For this purpose, 188 ecstasy-naïve volunteers (77M, 111F,  $21.7 \pm 3.0$  years) with high probability of first ecstasy use were examined at baseline. After a mean period of about 17 months, 59 incident ecstasy users ( $6.0 \pm 11.6$  tablets) and 56 matched persistent ecstasy-naïves were reexamined. Main outcome measures were serotonin transporter densities measured with [ $^{123}\text{I}$ ]β-CIT SPECT as indicator of serotonergic function; brain metabolites measured with  $^1\text{H}$ -MR spectroscopy as indicators of neuronal damage; apparent diffusion coefficient (ADC) and fractional anisotropy (FA) of the diffusional motion of water molecules in the brain measured with diffusion tensor imaging (DTI) as indicators of axonal integrity; regional relative cerebral blood volume (rrCBV) measured with perfusion weighted imaging indicative of brain perfusion. With this approach both structural ( $^1\text{H}$ -MRS and DTI) and functional ([ $^{123}\text{I}$ ]β-CIT SPECT and PWI) aspects of neurotoxicity are combined.

## Results

Compared to persistent ecstasy-naïve subjects, novel low-dose ecstasy users (mean 6.0 tablets; median 2.0 tablets) showed a decrease in brain perfusion, decreased FA, and increased ADC in the basal ganglia (all  $p < 0.05$ , adjusted for baseline measures and confounders). No changes in serotonin transporter densities and neurometabolites were observed.

## Conclusions

The findings suggest sustained vasoconstriction and probably axonal damage due to low dosages of ecstasy. Although we do not know yet whether these effects are reversible or not, we cannot exclude that ecstasy even in low doses is neurotoxic to the brain.

## INTRODUCTION

After cannabis, ecstasy (3,4-methylenedioxymethamphetamine, MDMA) is the most commonly used illegal substance in most countries worldwide, especially among adolescents and young adults. Among the 15–24 age group in Europe, lifetime experience rates range from 0.4 % to 13 %, while last year use ranges from 0.3 % to 11 %<sup>1</sup>. Highest prevalence rates were reported in Czech Republic, Spain and the United Kingdom and rates are generally higher in males than in females. In the United States, lifetime experience with ecstasy use was reported in 13.1 % of the 18- to 25-year olds, with last year use in 3.1 %<sup>2</sup>.

Despite its popularity, there are strong indications from animal and human studies that ecstasy is neurotoxic to the axons of serotonin cells<sup>3,4</sup>. Serotonin is an important neurotransmitter for regulation of many processes such as mood, memory and pain. However, results from animal studies cannot simply be extrapolated to humans, and most human studies are littered with methodological problems, including inadequate sampling of subjects and controls, lack of drug-use analysis, retrospective designs, lack of baseline data before first ecstasy use, and inclusion of only moderate or heavy ecstasy users<sup>5</sup>. This leaves the possibility that observed differences between ecstasy users and controls were biased or pre-existent, and explains why the discussion whether ecstasy is really neurotoxic in humans is ongoing<sup>6</sup>. It may also explain why it has been advocated to study the potential benefits of MDMA as an adjuvant in psychotherapy<sup>7</sup>. Few prospective studies examined the effects of ecstasy in volunteers without prior ecstasy experience, but these studies focused on acute and not on sustained or persistent effects of ecstasy on the brain<sup>8</sup>.

Only a long-term prospective study in ecstasy-naive individuals randomly assigned to MDMA or placebo can determine decisively whether recreational ecstasy use is neurotoxic in humans. Given the existing data on the potential neurotoxicity of ecstasy, such a study is ethically disputable. Therefore, it has recently been advocated to start prospective studies in specific groups with increased risk for future ecstasy use and re-examine these volunteers over years<sup>9</sup>. The current study, part of the Netherlands XTC Toxicity (NeXT) study, is the first that succeeded in this approach.

We prospectively assessed parameters of neurotoxicity of ecstasy in 59 volunteers before and after their first period of ecstasy and compared these novel ecstasy users with a matched group of 58 persistent ecstasy-naive volunteers. The parameters included [<sup>123</sup>I]β-CIT single photon emission computed tomography (SPECT) measuring serotonin transporter (SERT) densities; proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) measuring the neurometabolites N-acetylaspartate (NAA; neuronal marker), choline (Cho; reflecting cellular density) and myo-inositol (mI; marker for gliosis) relative to (phospho)creatine (Cr)<sup>10-12</sup>; diffusion tensor imaging (DTI) measuring apparent diffusion coefficient (ADC) and fractional anisotropy

(FA) of the diffusional motion of water molecules in the brain as indicators of axonal integrity<sup>13,14</sup>; and perfusion weighted imaging (PWI) measuring regional relative cerebral blood volume (rrCBV) indicative of brain perfusion<sup>15,16</sup>. With this approach we combined imaging techniques that measure both structural (<sup>1</sup>H-MRS and DTI) and functional ([<sup>123</sup>I]β-CIT SPECT and PWI) aspects of neurotoxicity. These parameters were previously used to measure neuronal damage in ecstasy users and in neuropsychiatric disorders. Based on previous findings in ecstasy users<sup>4,17-21</sup>, we hypothesized that if ecstasy is neurotoxic, novel ecstasy users, and not persistent ecstasy-naives, would show a decrease (after a short period of abstinence from ecstasy) or increase (after longer period abstinence from ecstasy) in rrCBV and ADC; an increase in brain metabolite ratios of choline and myo-inositol relative to creatine (Cho/Cr and mI/Cr); a decrease in [<sup>123</sup>I]β-CIT binding to SERTs; and a decrease in FA and the ratio of N-acetylaspartate to creatine (NAA/Cr).

## MATERIAL AND METHODS

### Design and subjects

A cohort of 188 ecstasy-naive young adults (77M, 111F, 21.7 ± 3.0 years) with a relatively high probability to start using ecstasy in the near future was recruited between April 2002 and April 2004 using a combination of targeted site sampling, advertisement and snowball sampling, described in a special design paper on the NeXT study<sup>22,23</sup>. Main inclusion criteria were high probability to use ecstasy in the near future, indicated by the intention to probably or certainly use ecstasy for the first time in the near future and/or having one or more friends who already used ecstasy. Exclusion criteria were age below 18 or above 35 years, ecstasy use in the past, severe physical or mental illness, use of psychotropic medications (e.g. serotonin reuptake inhibitors), pregnancy, and use of intravenous drugs. Subjects had to abstain from psychoactive substances for at least two weeks and from alcohol for at least one week before examinations. This was checked in urine (enzyme-multiplied immunoassay for amphetamines, MDMA, opioids, cocaine, benzodiazepine, cannabis, and alcohol). None of the subjects had to be excluded because of a positive urine drug test.

At baseline examination all subjects underwent brain imaging including SPECT and MR imaging. Thereafter, subjects were sent four questionnaires by mail regarding their drug use during a follow-up period of 12-24 months. Between 12 and 36 months after baseline assessments, all novel ecstasy users and an individually matched (gender, age, verbal intelligence, cannabis use) control group of persistent ecstasy-naives (subjects from the same baseline population who did not start to use

ecstasy during follow-up) were invited for a follow-up session during which brain imaging was repeated.

Subjects were paid for their participation (per session € 100 - €150). The study was approved by the local medical ethics committee and informed consent of each subject was obtained according to the Declaration of Helsinki. A subgroup of the prospective cohort underwent an intermediate session with MRI and psychopathology measurements soon after their first ecstasy use<sup>24</sup>. Subjects also completed questionnaires on psychopathology<sup>23</sup>, and underwent functional MRI and cognitive testing<sup>25</sup>, which are/will be reported in separate publications.

## Assessment of potential confounders

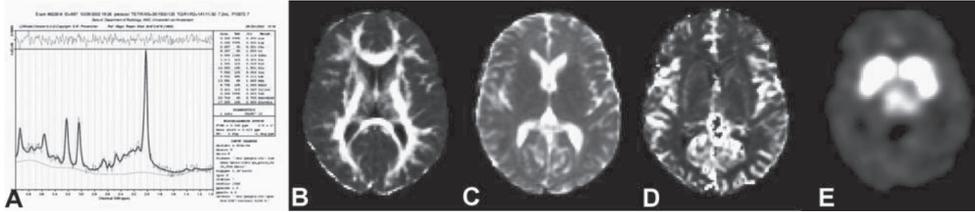
Potential confounders, such as demographic variables, education, and substance use were measured at baseline and follow-up. Aspects of lifetime ecstasy use (frequency of use, cumulative number of tablets, and duration of use), and last year use of alcohol (units per week), tobacco (cigarettes per week), cannabis (number of joints last year), amphetamines (number of times used last year), and cocaine (number of times used last year) were assessed using validated questionnaires<sup>26</sup>. An estimate of verbal intelligence was measured using The Dutch Adult Reading Test (DART), the Dutch version of the National Adult Reading Test<sup>27</sup>. Genotyping of the SERT was performed using polymerase chain reaction as described elsewhere<sup>28</sup>. Genotyping was successful in 93 out of 115 samples, because of poor DNA isolation in the others.

## MRI acquisition and post-processing

*Acquisition:* MRIs were performed on a 1.5 T scanner (Signa Horizon, LX 9.0, General Electric Medical Systems, Milwaukee, WI) using the standard head coil. MRI acquisition, post-processing and quality control were performed with the same methods used in a previous sub-study of the NeXT study<sup>24</sup>. For completeness, we summarize the most relevant aspects of the employed methods. The protocol included axial PD- and T2-weighted imaging; three voxel-based proton <sup>1</sup>H-MRS scans; DTI; PWI; and high resolution T1-weighted 3D imaging. The <sup>1</sup>H-MRS voxels were placed in the left centrum semiovale (frontoparietal white matter) and in mid-frontal and mid-occipital grey matter in analogy to previous studies<sup>17,20</sup>. Positioning of subjects, slices and voxels were performed by the same examiner and according to a protocol in order to keep positioning as reproducible as possible.

*Post-processing:* From the <sup>1</sup>H-MRS, ratios of NAA/Cr, Cho/Cr and mI/Cr were analyzed using LCModel (Linear Combination of Model spectra)<sup>29</sup>. ADC and FA maps were calculated from the DTI scans<sup>30</sup> and cerebral blood volume (CBV) maps from the PWI scans. FA, ADC and CBV were spatially normalized by registration

to the Montreal Neurological Institute brain template (MNI152) (Figure 1) and segmentation was performed into CSF, white and grey matter. The CBV maps were intensity-scaled to mean individual CBV intensity of white matter derived from the segmentation procedure to generate relative CBV (rCBV) maps.



**Figure 1:** Representative images of an individual (A)  $^1\text{H}$ -MRS after analysis by LCModel and representative (B) FA, (C) ADC, (D) rrCBV and (E)  $^{123}\text{I}$ β-CIT binding images after transformation to the spatially normalized MNI brain template.

Regions of interest (ROIs) were drawn on the MNI152 brain template in thalamus, putamen, globus pallidus, head of the caudate nucleus, centrum semiovale (frontoparietal white matter), and dorsolateralfrontal, mid-frontal, occipital, superior parietal, and temporal cortex. Only grey matter voxels were included in the cortical ROIs, whereas both white and grey matter voxels were included in the ROIs of the basal ganglia (i.e., CSF voxels were excluded). Selection of ROIs was based on findings of previous studies, which indicated that ecstasy-induced abnormalities are most prominent in basal ganglia and certain cortical areas; ecstasy-induced abnormalities in white matter were rarely reported and thus not expected. As cortical grey matter has very low anisotropy, it is very difficult to get reliable FA and ADC measurements in cortical areas. For this reason only white matter and basal ganglia ROIs were taken into account in the measurements of FA and ADC. Within the ROIs, individual mean values of FA, ADC, and regional relative CBV (rrCBV) were calculated. Values of FA, ADC and rrCBV from ROIs in left and right hemispheres were averaged.

## SPECT acquisition and post-processing

*Acquisition:* SPECT imaging was performed with the radioligand  $^{123}\text{I}$ β-CIT that binds non-selectively to SERTs. The procedure of radiosynthesis of  $^{123}\text{I}$ β-CIT and acquiring of SPECT images were the same as previously described<sup>31</sup>. SPECT images were acquired 4 h after the injection, when stable specific binding uptake to the SERTs is reached<sup>32</sup>.

*Post-processing:* Attenuation correction of all images was performed as previously described<sup>33</sup>. Images were reconstructed in 3D-mode (<http://www.neurophysics.com>). First, all SPECT scans were registered to the T1-3D MRI scans of the same

person by maximizing mutual information and applying rigid transformations using self-developed software. Second, the individual MRIs were registered to the 152MNI brain using affine transformation and these transformations were applied to register the individual SPECT scans to the 152MNI brain template (Figure 1). This resulted in  $91 \times 109 \times 91$  voxel images, with voxel sizes of  $2 \times 2 \times 2 \text{ mm}^3$ . For quantification, both ROI and voxel-by-voxel analyses were performed. For the ROI analysis, regions were drawn on the 152MNI template in midbrain, thalamus, temporal cortex, frontal cortex, and occipital cortex. We did not measure SERT uptake in the putamen, caudate nucleus and globus pallidus, because there is no specific binding to SERT or DAT in these regions 4 h after [ $^{123}\text{I}$ ] $\beta$ -CIT injection. Activity in the cerebellum was assumed to represent non-displaceable activity (nonspecific binding and free radioactivity). Specific to non-specific binding ratios were calculated as (activity in ROI – activity in cerebellum)/ activity in cerebellum. Image registration steps were visually inspected to check the quality.

Voxel-by-voxel analysis was performed with Statistical Parametric Mapping software (SPM2, Wellcome Department of Imaging Neuroscience, Functional Imaging Laboratory, London, UK; [www.fil.ion.ucl.ac.uk/spm](http://www.fil.ion.ucl.ac.uk/spm))<sup>34</sup>. The registered scans were intensity scaled to the corresponding mean cerebellar non-specific counts per voxel. The mean cerebellar counts were obtained from the ROI analysis. Then, smoothing was applied with SPM2 (Gaussian kernel with a 16 mm FWHM) to reduce interindividual anatomical differences that remained after stereotactical normalization<sup>35</sup>. Finally, difference images were created by subtracting the follow-up images from the baseline images.

## Statistical analyses

Substance-use variables were log-transformed before the analysis because they were not normally distributed. Future ecstasy use, i.e., ecstasy use between baseline and follow-up, was categorized as a binary variable: novel ecstasy user versus persistent ecstasy-naive volunteer. Baseline differences between novel ecstasy users and persistent ecstasy-naives in gender and SERT polymorphism were analyzed with Chi-Square tests and age, DART-IQ, years of education, and imaging parameters with the Student t-tests (two-sided).

To test whether ecstasy use had an effect on imaging parameters, separate linear regression analyses were performed with the follow-up outcomes as dependent variables, ecstasy use (yes/no) as the independent variable and baseline imaging outcomes as covariates (model 1). If the effect of novel ecstasy use in model 1 was statistically significant, a second linear regression analysis (enter) was performed in which the observed relationship was adjusted for the effect of potential confounders, i.e. gender, baseline verbal IQ and follow-up measures of age, years of education,

and the use of alcohol, cannabis, amphetamines, cocaine, and tobacco (model 2). In order to test whether changes in imaging parameters were related to the amount of ecstasy use, linear regression analyses were performed in the group of novel ecstasy users with follow-up outcomes as dependent variables, cumulative number of ecstasy tablets (log-transformed) as independent variable and baseline imaging parameters and potential confounders as covariates (model 3).

ROI analyses were performed using SPSS 11.5 (SPSS Inc., Chicago, IL, USA). Mean values reported in the result and discussion sections are followed by their standard deviations (mean  $\pm$  SD). Regression coefficients *B* are reported with 95% confidence intervals (95% CI) in the tables and in the text with their two-tailed significance level (*p*-values).

For the SPECT voxel-by-voxel analysis, difference images from the novel ecstasy users and the persistent ecstasy-naïves were compared on a voxel-by-voxel basis by means of the spatial extent statistical theory using SPM2<sup>34,35</sup>. The PET/SPECT model ‘compare-populations’ was chosen (two-sample *t*-test). An effect was considered statistically significant if a cluster of at least 20 connected voxels reached the one-sided *p*-value  $< 0.001$  ( $T = 3.30$ , uncorrected for multiple comparisons).

**Table 1:** Demographics, characteristics of ecstasy use and use of other substances. Values expressed as mean  $\pm$  SD, outcomes as regression coefficients (95% CI).

	Baseline		
	Persistent ecstasy naïves ( <i>N</i> = 56)	Future ecstasy users ( <i>N</i> = 59)	Mean difference (95% CI) <sup>a</sup>
Gender	23M, 33F	25M, 34F	<i>p</i> =0.888 <sup>b</sup>
Age	21.5 $\pm$ 2.1	21.7 $\pm$ 3.1	0.24 (-0.71; 1.19)
DART-IQ	105.3 $\pm$ 10.2	103.5 $\pm$ 9.0	-1.56 (-4.98; 1.87)
years of education	14.4 $\pm$ 1.8	13.9 $\pm$ 2.7	-0.45 (-1.26; 0.37)
SERT polymorphism ( <i>N</i> = 93)	15l,15l/s,16s	18l,21l/s,8s	<i>p</i> = 0.140 <sup>b</sup>
<b>Ecstasy</b>			
Cumulative dose (tablets)	NA	NA	
Time since first tablet (weeks)	NA	NA	
Time since last tablet (weeks)	NA	NA	
Duration of ecstasy use (weeks)	NA	NA	
<b>Other substances (last year)</b>			
Alcohol (units/week)	10.5 $\pm$ 9.1	8.6 $\pm$ 7.7	-0.24 (-0.59; 0.10)
Tobacco (cig/week)	25.8 $\pm$ 54.4	33.4 $\pm$ 47.5	0.85 (-0.01; 1.71)
Cannabis (joints in last year)	17.6 $\pm$ 25.4	48.0 $\pm$ 99.8	0.67 (-0.02; 1.37)
Amphetamine (number of times used last year)	0.0 $\pm$ 0.0	0.1 $\pm$ 0.8	0.04 (-0.04; 0.13)
Cocaine (number of times used last year)	0.4 $\pm$ 1.6	0.9 $\pm$ 2.2	0.21 (-0.09; 0.51)

## RESULTS

### Characteristics of the sample and substance use

Of the 188 volunteers at baseline, 158 (84%) completed the follow-up drug-use questionnaires. The other 30 volunteers (16%) either refused to participate in follow-up or were lost to follow-up. Of the 158 subjects, 64 (41%) started to use ecstasy during follow-up, whereas the other 94 subjects (59%) remained ecstasy-naive. Of the 64 novel ecstasy users, 59 (92%) agreed to participate in the follow-up session, together with 56 individually-matched persistent ecstasy-naive subjects, resulting in 115 subjects with a follow-up session. Time between baseline and follow-up measurements was  $15.9 \pm 4.6$  months in the ecstasy group and  $18.3 \pm 6.5$  months in the control group ( $p = 0.024$ ).

Table 1 shows baseline and follow-up sociodemographics and substance use data of subjects that participated in the follow-up session. During the follow-up period, novel ecstasy users used 6.0 tablets on average (range: 0.5 to 80; median 2.0 tablets) in a mean period of  $20.4 \pm 23.8$  weeks. At baseline, the two groups did not significantly differ in terms of gender, age, verbal IQ, SERT polymorphism, alcohol use, smoking, and use of cannabis, amphetamine and cocaine ( $p > 0.05$ ). However, between baseline and follow-up, novel ecstasy users reported modestly higher levels of consumption of

	Follow-up		Regression coefficient <i>B</i> (95% CI) <sup>c</sup>
	Persistent ecstasy naives ( <i>N</i> = 56)	Incident ecstasy users ( <i>N</i> = 59)	
	23.1 ± 2.1	23.0 ± 3.2	-0.20 (-0.38; -0.03)*
	15.9 ± 2.0	15.0 ± 2.8	-0.39 (-0.84; 0.05)
	NA	6.0 ± 11.6	
	NA	39.2 ± 23.4	
	NA	18.7 ± 17.5	
	NA	20.4 ± 23.8	
	8.7 ± 8.1	9.3 ± 8.6	0.59 (0.06; 1.11)*
	24.9 ± 46.1	39.6 ± 62.6	0.28 (-0.32; 0.88)
	21.1 ± 51.8	48.9 ± 114.2	0.59 (0.06; 1.11)*
	0.0 ± 0.0	0.6 ± 2.1	0.19 (0.00; 0.37)*
	0.4 ± 1.6	2.5 ± 7.3	0.43 (0.08; 0.79)*

<sup>a</sup> future ecstasy users vs persistent ecstasy-naives at baseline (*t*-test, substance use log-transformed)

<sup>b</sup> future ecstasy users vs persistent ecstasy-naives at baseline (Chi-square test)

<sup>c</sup> incident ecstasy users vs persistent ecstasy-naives at follow-up (linear regression adjusted for baseline scores, substance use log-transformed)

\* significant difference,  $p < 0.05$

**Table 2:** Results from [<sup>123</sup>I]β-CIT SPECT, 1H-MRS, DTI, and PWI. Expressed as mean ± SD. Scores are uncorrected for covariates.

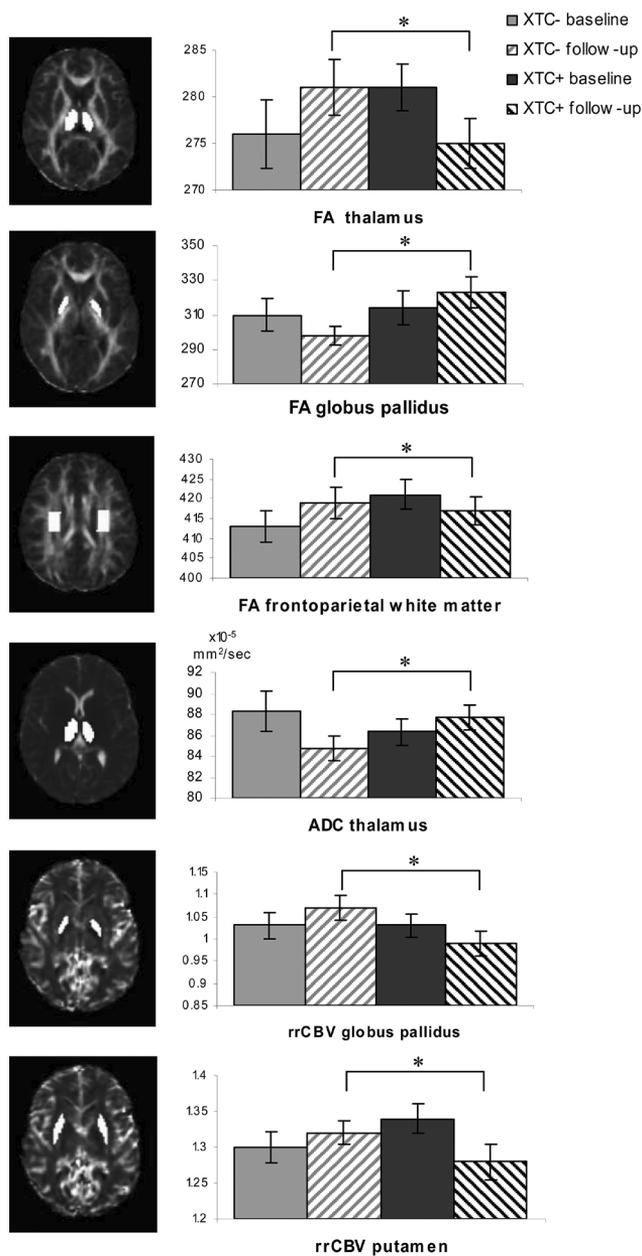
Imaging technique	Parameter	Region of interest	Baseline			
			Persistent ecstasy naïves	Future ecstasy users	Mean difference (95% CI) <sup>a</sup>	
SPECT (N <sub>xtc-naïves</sub> =53) (N <sub>(future)xtc</sub> =57)	[ <sup>123</sup> I]β-CIT binding ratios	midbrain	1.30 ± 0.31	1.33 ± 0.34	0.032 (-0.090; 0.154)	
		thalamus	1.26 ± 0.33	1.24 ± 0.29	-0.016 (-0.132; 0.101)	
		frontal grey matter	0.03 ± 0.14	0.07 ± 0.12	0.041 (-0.008; 0.089)	
		occipital grey matter	0.06 ± 0.11	0.05 ± 0.13	-0.006 (-0.051; 0.038)	
		temporal grey matter	0.32 ± 0.14	0.32 ± 0.15	0.000 (-0.055; 0.055)	
1H-MRS (N <sub>xtc-naïves</sub> =51) (N <sub>(future)xtc</sub> =52)	NAA/Cr	mid-frontal grey matter	1.30 ± 0.22	1.25 ± 0.25	-0.053 (-0.143; 0.037)	
		mid-occipital grey matter	1.49 ± 0.24	1.50 ± 0.18	0.015 (-0.067; 0.097)	
		left centrum semiovale	1.49 ± 0.30	1.49 ± 0.21	0.002 (-0.100; 0.103)	
	Cho/Cr	mid-frontal grey matter	0.24 ± 0.05	0.24 ± 0.04	-0.001 (-0.019; 0.017)	
		mid-occipital grey matter	0.15 ± 0.02	0.15 ± 0.02	-0.001 (-0.011; 0.008)	
		left centrum semiovale	0.33 ± 0.05	0.34 ± 0.04	0.011 (-0.008; 0.030)	
	mI/Cr	mid-frontal grey matter	0.90 ± 0.23	0.90 ± 0.15	-0.003 (-0.079; 0.073)	
		mid-occipital grey matter	0.79 ± 0.12	0.81 ± 0.10	0.015 (-0.028; 0.058)	
		left centrum semiovale	0.84 ± 0.16	0.83 ± 0.16	-0.012 (-0.074; 0.050)	
	DTI (N <sub>xtc-naïves</sub> =55) (N <sub>(future)xtc</sub> =56)	FA (x1000)	thalamus	276 ± 27	281 ± 19	5.30 (-3.39; 14.00)
			globus pallidus	310 ± 67	314 ± 72	4.44 (-21.79; 30.67)
			putamen	222 ± 36	224 ± 46	1.94 (-13.72; 17.59)
caudate nucleus			172 ± 34	176 ± 43	4.48 (-10.15; 19.12)	
centrum semiovale			413 ± 29	421 ± 27	7.83 (-2.77; 18.42)	
ADC 10-5 mm <sup>2</sup> /sec		thalamus	88.3 ± 14.2	86.3 ± 9.4	-2.05 (-6.58; 2.48)	
		globus pallidus	71.9 ± 2.3	72.1 ± 3.6	0.22 (-0.93; 1.37)	
		putamen	70.5 ± 1.7	70.7 ± 1.9	0.23 (-0.45; 0.92)	
		caudate nucleus	92.5 ± 15.2	91.6 ± 15.0	0.86 (-6.54; 4.82)	
		centrum semiovale	69.9 ± 2.2	70.3 ± 1.9	0.38 (-0.40; 1.15)	
PWI (N <sub>xtc-naïves</sub> =54) (N <sub>(future)xtc</sub> =54)	rrCBV	thalamus	1.63 ± 0.18	1.64 ± 0.20	0.005 (-0.068; 0.079)	
		globus pallidus	1.03 ± 0.22	1.03 ± 0.18	0.009 (-0.068; 0.086)	
		putamen	1.30 ± 0.15	1.34 ± 0.15	0.036 (-0.021; 0.094)	
		caudate nucleus	1.25 ± 0.16	1.28 ± 0.14	0.029 (-0.027; 0.085)	
		dorsolateral frontal grey matter	1.67 ± 0.14	1.65 ± 0.18	-0.033 (-0.095; 0.029)	
		mid-frontal grey matter	1.73 ± 0.17	1.69 ± 0.19	-0.041 (-0.111; 0.029)	
		occipital grey matter	2.13 ± 0.21	2.08 ± 0.31	-0.045 (-0.145; 0.055)	
		superior parietal grey matter	1.97 ± 0.18	1.93 ± 0.23	-0.032 (-0.112; 0.048)	
		temporal grey matter	2.03 ± 0.20	2.00 ± 0.21	-0.026 (-0.105; 0.053)	

<sup>a</sup> future ecstasy users vs persistent ecstasy-naïves at baseline (*t*-test)

<sup>b</sup> incident ecstasy users vs persistent ecstasy-naïves at follow-up (linear regression adjusted for baseline scores)

\* significant difference, *p* < 0.05

Follow-up		
Persistent ecstasy naives	Incident ecstasy users	Regression coefficient <i>B</i> (95% CI) <sup>b</sup>
1.27 ± 0.29	1.32 ± 0.32	0.036 (-0.068; 0.140)
1.12 ± 0.22	1.11 ± 0.21	-0.001 (-0.077; 0.076)
0.00 ± 0.08	0.01 ± 0.10	-0.003 (-0.036; 0.31)
0.05 ± 0.09	0.03 ± 0.10	-0.018 (-0.051; 0.015)
0.28 ± 0.12	0.27 ± 0.14	-0.009 (-0.053; 0.35)
1.27 ± 0.20	1.29 ± 0.18	0.015 (-0.059; 0.089)
1.52 ± 0.24	1.50 ± 0.19	-0.022 (-0.108; 0.064)
1.46 ± 0.17	1.50 ± 0.22	0.043 (-0.032; 0.117)
0.23 ± 0.05	0.25 ± 0.05	0.018 (-0.001; 0.038)
0.15 ± 0.02	0.15 ± 0.02	-0.002 (-0.010; 0.006)
0.34 ± 0.04	0.35 ± 0.05	0.004 (-0.011; 0.018)
0.88 ± 0.18	0.89 ± 0.18	0.006 (-0.063; 0.076)
0.83 ± 0.13	0.81 ± 0.11	-0.023 (-0.071; 0.025)
0.86 ± 0.17	0.84 ± 0.14	-0.019 (-0.077; 0.038)
<b>281 ± 22</b>	<b>275 ± 20</b>	<b>-8.16 (-15.30; -1.02)*</b>
<b>298 ± 38</b>	<b>323 ± 70</b>	<b>23.19 (3.65; 42.73)*</b>
221 ± 29	224 ± 38	2.63 (-7.87; 13.14)
173 ± 38	173 ± 37	-2.26 (-11.78; 7.26)
<b>419 ± 30</b>	<b>417 ± 26</b>	<b>-8.23 (-14.35; -2.10)*</b>
<b>84.8 ± 8.7</b>	<b>87.7 ± 9.3</b>	<b>3.68 (0.67; 6.69)*</b>
71.8 ± 2.3	72.1 ± 3.5	0.13 (-0.81; 1.07)
70.5 ± 1.6	70.5 ± 1.8	-0.12 (-0.71; 0.48)
91.51 ± 15.6	93.3 ± 14.3	2.74 (-1.76; 7.25)
69.9 ± 2.1	70.4 ± 1.8	0.22 (-0.26; 0.70)
1.59 ± 0.20	1.62 ± 0.19	0.028 (-0.041; 0.097)
<b>1.07 ± 0.20</b>	<b>0.99 ± 0.21</b>	<b>-0.083 (-0.154; -0.012)*</b>
<b>1.32 ± 0.12</b>	<b>1.28 ± 0.18</b>	<b>-0.058 (-0.110; -0.006)*</b>
1.23 ± 0.17	1.28 ± 0.18	0.028 (-0.029; 0.085)
1.70 ± 0.13	1.65 ± 0.17	-0.040 (-0.091; 0.011)
1.72 ± 0.21	1.69 ± 0.19	-0.007 (-0.068; 0.055)
2.11 ± 0.23	2.08 ± 0.29	-0.009 (-0.097; 0.078)
1.99 ± 0.19	1.94 ± 0.24	-0.033 (-0.105; 0.039)
2.03 ± 0.18	2.00 ± 0.26	-0.010 (-0.087; 0.068)



**Figure 2:** On the left FA, ADC and rCBV maps with brain regions (marked in white) significantly different between novel ecstasy users and persistent ecstasy-naïves at follow-up, corrected for baseline measurements. On the right corresponding FA values in the thalamus, globus pallidus and frontoparietal white matter, ADC values in the thalamus, and rrCBV values in the globus pallidus and the putamen in ecstasy-naïves (XTC -) and novel ecstasy users (XTC +) at baseline and at follow-up. Results represent mean  $\pm$  SEM; \* =  $p < 0.05$  at follow-up, corrected for baseline measurements. Baseline scores were not significantly different between both groups ( $p > 0.05$ ). Only significant results are shown, for complete results of all analyses see Table 2. Note that the vertical axis does not start at zero.

alcohol ( $p = 0.036$ ), cannabis ( $p = 0.029$ ), amphetamines ( $p = 0.047$ ), and cocaine ( $p = 0.017$ ) than persistent ecstasy-naive controls.

## Brain imaging

The SPECT scans of one subject had to be excluded because of mis-registration to the standard brain. In addition, due to incidental technical problems with the scanners and production of [ $^{123}\text{I}$ ] $\beta$ -CIT or the refusal of a subject for a particular part of the study, we do not have complete baseline and follow-up data sets of each subject for all imaging modalities. The numbers of complete datasets per modality are given in Table 2.

Table 2 also shows the results from baseline and follow-up comparisons. The two groups did not significantly differ on any of the neuroimaging parameters at baseline. Neither region of interest (ROI) nor voxel-by-voxel analysis showed any significant effect of ecstasy use on [ $^{123}\text{I}$ ] $\beta$ -CIT binding. Also no significant effect of ecstasy was observed on brain metabolite ratios. However, ecstasy users, relative to non-users, showed a significant decrease of FA in the thalamus ( $p = 0.025$ ) and frontoparietal white matter ( $p = 0.009$ ), and of rrCBV in the globus pallidus ( $p = 0.022$ ) and putamen ( $p = 0.029$ ), whereas they showed a significant increase of FA in the globus pallidus ( $p = 0.020$ ) and of ADC in the thalamus ( $p = 0.017$ ) (Figure 2). After correction for potential confounders such as the use of other substances (model 2), all effects of ecstasy according to model 1 remained significant. Within the group of novel ecstasy users, we found no significant dose-response effects of cumulative doses of ecstasy on follow-up outcomes (model 3).

## DISCUSSION

This first prospective imaging study in novel ecstasy users suggest that even a low to moderate ecstasy dose of 1-80 tablets (mean 6 tablets; median 2 tablets) has sustained effects on the brain as indicated by differences between novel ecstasy users and persistent ecstasy-naive controls in FA, ADC and rrCBV. An intermediate follow-up session with MRI in a sub-sample of the same population quite soon after their first ecstasy use ( $N = 30$ , mean of 1.8 ecstasy tablets, without ecstasy-naive controls) also showed some changes in MR imaging parameters, although outcomes of some parameters differ from the current study<sup>24</sup>. The data from the current study are superior, because of a larger study population, higher cumulative ecstasy dose, comparisons with persistent ecstasy-naive controls, and inclusion of [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT.

Decreased FA and increased ADC in the thalamus may reflect ecstasy-induced axonal damage, because axonal cell membranes are known to be responsible for most of the restriction of water diffusion<sup>14</sup> and axonal damage lead to decreased FA and increased ADC. This finding of ecstasy-induced brain pathology in the thalamus corroborates findings from previous studies showing decreased thalamic SERT densities in (heavy) ecstasy users, most probably reflecting damage to the terminals of serotonergic axons<sup>36,37</sup>. A study in rats even showed large numbers of degenerating axons in the thalamus after a single exposure to MDMA<sup>38</sup>. Moreover, as the thalamus is important for neurocognitive processes, one can speculate that ecstasy-induced thalamic damage is (partly) responsible for decreased verbal memory performance frequently reported in heavy ecstasy users<sup>39,40</sup> and recently also shown in the current prospective cohort of low dose ecstasy users<sup>25</sup>. Decreased FA in the frontoparietal white matter may also reflect axonal injury. A previous study among heavy ecstasy users showed increased myo-inositol in the same area correlated with cumulative lifetime ecstasy dose, probably reflecting gliosis<sup>18</sup>, although animal studies have shown that ecstasy mainly affects the cortex and basal ganglia. Our data suggest that the significant difference in FA values between novel ecstasy users and persistent ecstasy-naïve subjects results from a combination of a relative decrease in FA in novel ecstasy users and an increase in FA in persistent ecstasy-naïves during the follow-up period (Figure 2). A previous study showed a positive relation between mean FA and age in young adults between 20.7 and 39.5 years, suggesting that structural changes in the brain continue in normal young adults, especially in areas with low anisotropy<sup>41</sup>. Therefore, we speculate that this normal maturation did occur in the persistent ecstasy-naïves, whereas the increase in FA failed to occur in the group of novel ecstasy users.

In contrast, we observed an increase in FA in the globus pallidus of novel ecstasy users relative to persistent ecstasy-naïves. Increased anisotropy was previously observed in acute stroke<sup>42</sup>. However, the increased FA in the globus pallidus may also be related to the decreased rrCBV values observed in the globus pallidus and putamen in the current study. Decreased rrCBV may reflect a prolonged vasoconstriction after ecstasy use, resulting in a decrease in extracellular water content due to ecstasy-induced changes in the serotonergic regulation of the microcirculation<sup>43</sup>. A previous SPECT study observed sub-acute decreases in CBF (cerebral blood flow) after only two doses of MDMA, 2-3 weeks after ecstasy intake<sup>20</sup>. The mean period of abstinence in our present study was 18.7 weeks, and therefore sub-acute ecstasy-induced vasoconstriction seems unlikely. Moreover, in a previous study we observed *increased* rrCBV and ADC in the globus pallidus of heavy ecstasy users with an average abstinence period of 14.6 weeks and suggested that this was caused by ecstasy-induced serotonergic depletion<sup>44</sup>. We also showed a positive correlation between 5-HT<sub>2</sub> receptor densities and rrCBV values in ecstasy users<sup>21</sup>. These apparent inconsistencies suggest there is a complex,

yet to be unraveled, relationship between ecstasy use and serotonergic-mediated brain perfusion, probably related to time since last tablet, cumulative dose, and adaptation of serotonergic transporters and receptors to the ecstasy-induced increase of serotonin in the (sub)acute stage and serotonergic depletion on the long-term.

We did not observe changes in SERT densities and brain metabolites before and after first ecstasy use. Various studies showed that reductions in SERTs are dose dependent and observed no differences between moderate ecstasy users and controls<sup>4</sup>. The same holds true for previous <sup>1</sup>H-MRS studies that showed decreased NAA and increased mI metabolite ratios in heavy users<sup>17,18,45</sup>, whereas others found no change in metabolite ratios in subjects with more moderate lifetime doses<sup>19</sup>. The current observation that low dose ecstasy use has no effect on central SERTs and brain metabolites is thus in line with previous studies in moderate ecstasy users.

No significant differences in substance use, SERT polymorphisms, SERT densities, and other imaging parameters were found between future ecstasy users and persistent ecstasy-naïves at baseline. This is an important finding, because it has been repeatedly suggested that observed differences between ecstasy users and non-users in previous studies may have been a pre-existing risk factor for ecstasy use rather than a neurotoxic consequence of this drug.

We are aware of some methodological limitations of our study, some of which have been discussed previously<sup>23</sup>. This study provides information about the relationship between ecstasy use and imaging parameters, but offers no undisputable evidence of causality. First, although prospective, the study design was not experimental, and no dose response relationship was found. Therefore, it is possible that the observed changes are not related to ecstasy use, but to other time- or ecstasy-related variables. Confounding by other substances cannot be totally excluded, although the findings remained significant after adjusting for differences in use of these substances. Second, there was no control on purity and the amount of MDMA in the ecstasy tablets, although more than 95% of the tablets sold as ecstasy in the Netherlands contains MDMA as the main component<sup>46</sup>. Third, the pattern of use and the setting in which ecstasy was taken were not taken into account. Fourth, the study mainly comprised low-dose ecstasy users. Because expected neuronal damage after a low dose of ecstasy is relatively minor, the statistical power of this study may have been insufficient for [<sup>123</sup>I]β-CIT SPECT and <sup>1</sup>H-MRS to detect existing changes. Fifth, since few studies used <sup>1</sup>H-MRS, DTI, and PWI to study neuronal damage in ecstasy users, little is known about sensitivity and specificity of these techniques to detect ecstasy-induced neuronal damage. Therefore, additional studies are needed, both in animals and in heavy human ecstasy users. On the other hand, <sup>1</sup>H-MRS, DTI and PWI have been shown to be sensitive tools in various neuropsychiatric disorders<sup>47-49</sup>, in substance users<sup>4,50</sup> and in detection of early diffuse axonal injury after traumatic brain injury<sup>51</sup>. Finally, because we studied early indicators of potential brain damage, and did not want to overlook such

indicators because of their social impact, we did not correct for multiple comparisons to minimize the risk of false negative results (type II errors)<sup>52</sup>. although this may have introduced some false positive findings (type I errors).

In conclusion, this prospective study showed certain measurable effects like prolonged vasoconstriction and some discrete but significant findings compatible with axonal damage even after low dose ecstasy use. Because we do not know yet whether these effects are permanent or reversible, we cannot conclude that ecstasy even in low doses is safe to the brain.

## ACKNOWLEDGEMENT

The NeXT study was supported by a grant of The Netherlands Organization for Health Research and Development as part of their Addiction Program (ZonMw 310-00-036). We thank Nick Ramsey, Dirk Korf, Hylke Vervaeke, Sarah Dijkink, Ivo Bisschops, Jacco Visser, Benoit Faivre, Dick Veltman, Matthan Caan, Frans Vos, Marcel van Herk, Jan Habraken, Erik-Jan Vlieger, Jeroen Snel, Charles Majoie, Ben Schmand and M. Moseley for help on the study design, recruiting volunteers, collecting data, post-processing, data analyses and review of the manuscript.

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CHAPTER  
12

# **Ecstasy Use and Self-Reported Depression, Impulsivity, and Sensation Seeking: a Prospective Cohort Study**

Journal of Psychopharmacology 2006; 20: 226-235

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# ABSTRACT

## Background

Although there are indications that ecstasy users have higher levels of depression, impulsivity, and sensation seeking, it is unknown whether these are consequences of ecstasy use or predisposing factors for starting ecstasy use.

## Aim

To prospectively assess the predictive value of depression, impulsivity, and sensation seeking on future first time ecstasy use. We also assessed whether depression, impulsivity, and sensation seeking had changed after first ecstasy use.

## Material and Methods

Depression, impulsivity, and sensation seeking were assessed using self-report questionnaires in 188 ecstasy-naive volunteers with high probability for future ecstasy use. After a mean follow-up of 17 months, measurements were repeated in 59 incident ecstasy users (mean 6.0 tablets) and 61 matched persistent ecstasy-naive volunteers.

## Results

Only experience seeking (subscale of the sensation seeking scale) predicted future ecstasy use (OR = 1.05, 95% CI 1.00 to 1.10), but after adjustment for potential confounders this was not significant anymore. At follow-up, significant effects of ecstasy use on the general and the disinhibition subscale of the sensation seeking scale were observed (after adjustment for potential confounders: regression coefficient  $B = 0.51$ , 95% CI 0.20 to 0.83 and  $B = 3.25$ , 95% CI 1.74 to 4.76, respectively).

## Conclusions

These data indicate that depression, impulsivity, and sensation seeking do not predict first time ecstasy use in a population of young adults with the intention to start using ecstasy and that low level ecstasy use does not seem to cause depression or impulsivity. However, low level ecstasy use may increase (certain aspects of) sensation seeking.

## INTRODUCTION

While the trend of using ecstasy (3,4-methylenedioxymethamphetamine, MDMA) has become increasingly widespread during the last decade, more concern has arisen about the negative consequences of the drug. The potential neurotoxicity of the party drug has been described in numerous articles over the last years. There is good evidence that MDMA causes damage to the serotonergic (5-HT) axons in animals, including rodents and non-human primates<sup>1</sup>. Also in humans there are indications that ecstasy use is associated with altered serotonergic function<sup>2-5</sup>. It is known that 5-HT modulates many physiological processes (such as vasoconstriction, thermoregulation) and neuropsychological functions (such as memory, and learning)<sup>6,7</sup>. Moreover, there is suggestive evidence that 5-HT function influences behavioural and psychopathological processes such as mood, anxiety, aggression, sexual behaviour, binge eating, sensation seeking, and impulsivity<sup>8-10</sup>. In line with this, depression was found to be associated with low 5-HT transporter densities<sup>11</sup>, high sensation seeking with low serotonergic function<sup>9</sup>, and impulsive and aggressive personality traits with low levels of the main serotonin metabolite 5-HIAA in the CSF<sup>12,13</sup>.

Therefore it can be hypothesized that ecstasy-induced serotonergic depletion will be associated with increased depression, impulsivity, and sensation seeking. In accordance with this hypothesis many studies reported increased self-reported levels of depression in recent and former ecstasy users<sup>2,14-20</sup>, which was confirmed by a recent meta-analysis<sup>21</sup>. In addition, ecstasy users were found to score higher on self-reported impulsivity<sup>22-28</sup> and sensation/novelty seeking<sup>2,24,29,30</sup>. In addition, the few studies that used behavioural measurements suggest increased depression by a shift towards negative words in the Affective Go/No-Go test<sup>31</sup>, increased behavioural impulsivity<sup>22</sup>, and increased risk-taking<sup>28</sup>. Others did not find differences on behavioural measurements such as decision-making<sup>32</sup>.

However, it is unclear, whether these associations reflect a causal relationship, i.e. whether the use of ecstasy causes changes in mood, impulsivity, and sensation seeking. Almost all previous studies were cross-sectional and thus vulnerable for selection and interpretation bias, including reverse causation. Moreover, many confounding variables are involved such as polydrug use, lifestyle, and education, that could account for these differences and it is very difficult or even impossible to control adequately for all these factors using a retrospective design<sup>33</sup>. It is conceivable that differences between ecstasy users and non-users were already existing before the first use of ecstasy or even predispose subjects to start using ecstasy<sup>34</sup>. Therefore, it has been advocated by several researchers that prospective studies should be conducted with measurements before and after a period of ecstasy use<sup>23,28</sup>.

A causal relationship between ecstasy-induced 5-HT dysfunction and increased depression and impulsivity is suggested by findings that increased impulsivity<sup>22</sup>

and depression<sup>20,21</sup> were positively correlated with increasing cumulative doses of ecstasy. Moreover, these studies compared ecstasy users with ecstasy-naive polydrug users and it seems implausible that individuals with high impulsivity or depression would be predisposed especially to the use of ecstasy. In rats, both MDMA<sup>35</sup> and cocaine<sup>36</sup> induced increased impulsive behaviour. On the other hand, other studies suggested that increased psychopathology is related to polydrug use rather than to the use of ecstasy alone<sup>19,25,26,37</sup>. Finally, there are studies which indicate that depression, impulsiveness, and personality traits such as sensation or novelty seeking are risk factors for substance use rather than a consequence of ecstasy use<sup>38,39</sup>, although few longitudinal studies in humans have been performed. One longitudinal follow-up study found that sensation seeking was a predictor for substance use<sup>40</sup>. Others observed that most (former) ecstasy users with increased depression scores reported that the onset of their mood disorder preceded their first use of ecstasy<sup>20,41</sup>. It has even been suggested that some individuals unconsciously chose to use ecstasy in an attempt to self-medicate their existing serotonergic deficiency<sup>2</sup>.

In summary, although there are indications that ecstasy users have higher levels of depression, impulsivity, and sensation seeking, it is not known whether these symptoms appear after the onset of ecstasy use and should be considered as a consequence of ecstasy use or whether these are pre-existing and could therefore be considered as predisposing or risk factors for ecstasy use. Only a prospective follow-up study in ecstasy-naive subjects can provide evidence of the direction of these relationships. Therefore the aim of the present study was to determine whether ecstasy users differ from ecstasy-naive subjects with regard to symptoms of depression, impulsivity, and sensation seeking and if so, whether differences were present before or developed after the first use of ecstasy. We hypothesized that higher levels of depression, impulsivity, and sensation seeking are predictors of future ecstasy use in ecstasy-naive young adults (even after controlling for potential confounders). In addition, we hypothesized that depression, impulsivity, and sensation seeking increase following a period of ecstasy use (even after controlling for potential confounders).

## METHODS AND MATERIALS

### Subjects

The current study is part of the NeXT (Netherlands XTC Toxicity) study, a larger study investigating causality, course, and clinical relevance of ecstasy neurotoxicity. A detailed description of the NeXT study can be found in a special design paper<sup>42</sup>. Between April 2002 and April 2004, 188 ecstasy-naive young adults (18-35 years) with a relatively high probability to start using ecstasy in the near future were included in

the study. They were actively recruited using a combination of targeted site sampling at locations such as dance events, discotheques, youth fairs, universities, colleges, and parks; advertisement through a website on the project and an internet campaign; and snowball sampling referrals. Main criteria for inclusion were intention to probably or certainly use ecstasy for the first time in the near future (a score of 3-5 on a 5-point scale; 1=certainly not; 2=probably not; 3=undecided; 4=probably yes; 5=certainly yes) and/or having one or more friends who already use ecstasy. Exclusion criteria were: ecstasy use in the past (at baseline session), a severe physical or mental illness, the use of psychotropic medications such as 5-HT reuptake inhibitors, pregnancy, and the use of intravenous drugs. Subjects had to abstain from use of psychoactive substances for at least two weeks before examinations and from alcohol for at least one week before examinations. This was checked in urine drug screening (enzyme-multiplied immunoassay for amphetamines, ecstasy, opiates, cocaine, benzodiazepine, cannabis, and alcohol). After recruitment we had about 550 volunteers who were willing to participate in the study, but the majority was excluded because their intention for future ecstasy use did not fulfil our criteria. A smaller part was excluded because of the other exclusion criteria or because they did not want to abstain from substance use. Subjects were paid for their participation (per session € 100,- or €150,- depending on measurements and location for 2 days of measurements including additional brain imaging; see also <sup>42</sup>).

The study was approved by the local medical ethics committee. To rule out any suggestion that we would approve or stimulate the use of ecstasy in ecstasy-naive subjects, subjects were informed through a brochure that stated “From research in animals it is shown that ecstasy potentially causes damage to the ‘serotonergic system’ in the brain. This system is important for several brain functions such as mood, impulsivity, and memory. However, we do not know whether this is also true for humans. Therefore it is important to study also in humans the potential consequences of ecstasy on the brain.” In addition each subject had to sign an informed consent form, that stated that participation was voluntary, that ecstasy is potential harmful and that the examiners do not have the intention to stimulate the use of ecstasy.

## Study procedure

At baseline examination all 188 subjects completed questionnaires regarding depression, impulsivity, and sensation seeking. After inclusion and baseline examination, subjects had to complete questionnaires (four in total) sent to them by mail about their drug use every three months during a follow-up period of approximately 18 months.

Between 12 and 24 months after the baseline assessments all incident ecstasy users and an individually matched (gender, age, verbal intelligence, cannabis use) control group of persistent ecstasy-naive subjects (subjects from the same baseline population

of ecstasy-naïves who did not start to use ecstasy during the follow-up period) were invited for a follow-up session during which the self-report questionnaires (depression, impulsivity, sensation seeking) were repeated.

## Assessments

### Depression

Current depressive symptoms were assessed using the Beck Depression Inventory (BDI) <sup>43</sup>. The BDI is a 21-item self-report rating inventory, which measures characteristic attitudes and symptoms of depression in the week prior to assessment; higher scores indicate more depressive symptoms. The BDI showed high levels of reliability and validity <sup>44,45</sup>. Total BDI scores were calculated.

### Impulsivity

The Dutch version of the Barratt Impulsiveness Scale (BIS-11), a consistent and reliable measure of impulsiveness, was used to assess impulsivity <sup>46</sup>. The Dutch BIS-11 contains 31 self-report items that have to be scored from 1 to 4. Total scores and subscale scores on attentional impulsivity ('difficulty in concentrating'), motor impulsivity ('acting without thinking') and non-planning impulsivity ('thinking about the present rather than the future') were calculated.

### Sensation Seeking

The Spannings Behoeftelijst (SBL), a Dutch adaptation of the American Sensation Seeking Scale <sup>47</sup>, was used to measure sensation seeking <sup>48,49</sup>. The SBL contains 51 sensation seeking items and 16 filler items, for which respondents have to indicate on a five-point scale to what extent they (dis)agree with the statements. Both a general sensation seeking score and scores for the subscales thrill and adventure seeking (TAS), experience seeking (ES), boredom susceptibility (BS) and disinhibition (DIS) were calculated. The (sub)scale scores have proven to be reliable measures for various aspects of sensation seeking in research populations <sup>48,49</sup>.

### Potential confounders

Potential confounders, such as demographic variables, education, and substance use were measured using questionnaires at baseline and at follow-up sessions. Various aspects of lifetime ecstasy use (frequency of use, cumulative number of tablets, and duration of use), and last year use of alcohol (units per week), tobacco (cigarettes per week), cannabis (number of joints last year), amphetamines (number of times used last year), and cocaine (number of times used last year) were assessed using validated substance-use questionnaires <sup>50</sup>. Verbal intelligence was measured using The Dutch Adult Reading Test (DART), the Dutch version of the National Adult Reading Test <sup>51</sup>. The DART-IQ

is used to estimate pre-morbid verbal intelligence as it is relatively insensitive to cognitive impairments caused by neurological disorders<sup>52</sup>.

## Statistical analyses

All substance-use variables were log-transformed before the analyses because they were not normally distributed. Future ecstasy use, i.e. ecstasy use between baseline and follow-up, was categorized in a binary variable: yes = 1 and no = 0.

To test whether higher levels of depression, impulsivity, and sensation seeking predicted future incident ecstasy use, backward logistic regression analyses ( $p_{in} = 0.05$ ;  $p_{out} = 0.05$ ) were performed. For significance testing Likelihood Ratio tests (LR tests) were applied. First, analyses were performed with future ecstasy use as the dichotomous dependent variable and baseline measures of the total scores on depression (BDI-BL), impulsivity (BIS-BL), and sensation seeking (SBL-BL) as the dependent variables, without (model 1, total scores) and with (model 2, total scores) adjustment for the effect of potential confounders. Second, to test whether certain aspects of impulsivity and sensation seeking predicted future ecstasy use, analyses were performed with future ecstasy use as the dichotomous dependent variable and baseline measures of the subscale scores on impulsivity (BISattentional-BL, BISmotor-BL, BISnon-planning-BL) and sensation seeking (SBL-TAS-BL, SBL-ES-BL, SBL-BS-BL, SBL-DIS-BL) as the dependent variables, also without (model 1, subscale scores) and with (model 2, subscale scores) adjustment for the effect of potential confounders. Following<sup>53</sup>, potential confounders (i.e. baseline measures of age, gender, verbal IQ, years of education, and substance use parameters) were defined as variables that were related to the dependent variable at the  $p < 0.20$  level of significance.

To test whether depression, impulsivity, and sensation seeking increased after a period of ecstasy use, separate linear regression analyses were performed with the follow-up total and subscale measures of depression (BDI-FU), impulsivity (BIS-FU), or sensation seeking (SBL-FU) as the dependent variables, ecstasy use as the independent variable and baseline measures of depression (BDI-BL), impulsivity (BIS-BL), or sensation seeking (SBL-BL) as covariates (model 1). In additional backward linear regression analyses, the observed relationships were adjusted for the effect of potential confounders, i.e. baseline measure of gender and verbal IQ and follow-up measures of age, years of education, and substance use parameters (model 2). For each analysis potential confounders were defined as variables that were related to the dependent variable at the  $p < 0.20$  level of significance<sup>53</sup>. In order to test whether the amount of ecstasy use was related to changes in depression, impulsivity, or sensation seeking, separate backward linear regression analyses were performed in the group of incident ecstasy users with follow-up measures of total and subscale

scores on depression (BDI-FU), impulsivity (BIS-FU), or sensation seeking (SBL-FU) as the dependent variable, the level of ecstasy use (cumulative number of tablets) as the independent variable and baseline scores on depression (BDI-BL), impulsivity (BIS-BL), or sensation seeking (SBL-BL) and potential confounders, related to the dependent variable at the level of  $p < 0.20$ , as covariates (model 3).

All analyses were performed using SPSS version 11.5; SPSS Inc., Chicago, IL, USA). Mean values reported in the result and discussion sections are followed by their standard deviations (mean  $\pm$  SD). Odds Ratios (OR) and regression coefficients  $B$  are reported with their 95% confidence intervals (95% CI).

## RESULTS

### Characteristics of the sample and substance use

Of the 188 ecstasy-naive subjects (77M, 111 F, mean age  $21.7 \pm 3.0$  yrs) at baseline, 158 (84%) completed the last follow-up questionnaires about drug use sent to them by mail. The other 30 volunteers (16%) were regarded as dropouts, either because they

**Table 1:** Demographics, characteristics of ecstasy use and use of other substances. Values expressed as mean  $\pm$  SD, outcomes as regression coefficients  $B$  (95% CI).

	Baseline		
	Persistent ecstasy naives ( $N = 61$ )	Future ecstasy users ( $N = 59$ )	Regression coefficient $B$ (95% CI) <sup>a</sup>
Gender	25M, 36F	25M, 34F	-0.01 (-0.19; 0.17)
Age	21.5 $\pm$ 2.0	21.7 $\pm$ 3.1	0.24 (-0.71; 1.19)
DART-IQ	105.1 $\pm$ 9.9	103.5 $\pm$ 9.0	-1.56 (-4.98; 1.87)
years of education	14.4 $\pm$ 1.8	13.9 $\pm$ 2.7	-0.45 (-1.26; 0.37)
<b>Ecstasy</b>			
Cumulative dose (tablets)	NA	NA	
Time since first tablet (weeks)	NA	NA	
Time since last tablet (weeks)	NA	NA	
Duration of ecstasy use (weeks)	NA	NA	
<b>Other substances (last year)</b>			
Alcohol (units/week)	9.9 $\pm$ 9.0	8.6 $\pm$ 7.7	-0.17 (-0.51; 0.17)
Tobacco (cig/week)	27.6 $\pm$ 55.3	33.4 $\pm$ 47.5	0.79 (-0.06; 1.64)
Cannabis (joints in last year)	17.6 $\pm$ 25.1	48.0 $\pm$ 100.0	0.69 (0.01-1.37)*
Amphetamine (number of times used last year)	0.0 $\pm$ 0.0	0.1 $\pm$ 0.8	0.04 (-0.04; 0.13)
Cocaine (number of times used last year)	0.4 $\pm$ 1.5	0.9 $\pm$ 2.8	0.22 (-0.07; 0.51)

<sup>a</sup> future ecstasy users vs persistent ecstasy-naives at baseline (linear regression, regression coefficient = mean difference, substance use log-transformed)

refused to participate in follow-up or because we could not reach them anymore. Of the 158 subjects, 64 (41%) declared they had started to use ecstasy since the inclusion in the study, while the other 94 subjects (59%) declared to be continuously ecstasy-naive. Of the 64 incident ecstasy users 59 (92%) were willing to participate in the follow-up session, together with 61 individually matched (gender, age, DART-IQ, cannabis use) persistent ecstasy-naive subjects, resulting in 120 subjects with a follow-up session. Time between baseline and follow-up measurements was on average  $16.0 \pm 4.6$  months (range: 11.5 - 35.5 months) in the ecstasy group and on average  $19.1 \pm 7.5$  months (range: 12.0 - 37.3 months) in the control group (mean difference 3.2 months, 95% CI 0.92 to 5.41).

Table 1 shows baseline and follow-up characteristics on demographics and substance use of subjects that participated in the follow-up session. It shows that incident ecstasy users used 6.0 tablets on average (range: 0.5 – 80; median 2.0 tablets) in a mean period of  $20.4 \pm 23.8$  weeks during the average follow-up period of 16.0 months. Table 1 also shows that due to the matching between the incident ecstasy users and the persistent ecstasy-naive subjects, the two groups were very similar in terms of gender, age, and verbal IQ prior to inclusion in the study. At baseline, the two groups were also similar in terms of alcohol use, smoking, and use of amphetamine and cocaine. However,

	Follow-up		Regression coefficient <i>B</i> (95% CI) <sup>b</sup>
	Persistent ecstasy naives ( <i>N</i> = 61)	Incident ecstasy users ( <i>N</i> = 59)	
	23.1 ± 2.1	23.0 ± 3.2	-0.28 (-0.46; -0.09)*
	15.9 ± 2.0	15.0 ± 2.8	-0.49 (-0.94; -0.05)*
	NA	6.0 ± 11.6	
	NA	39.2 ± 23.4	
	NA	18.7 ± 17.5	
	NA	20.4 ± 23.8	
	8.3 ± 7.9	9.4 ± 8.7	0.21 (0.02; 0.39)*
	25.7 ± 46.8	39.6 ± 62.6	0.26 (-0.32; 0.84)
	20.4 ± 49.9	48.9 ± 114.2	0.60 (0.09; 1.10)*
	0.0 ± 0.0	0.6 ± 2.1	0.19 (0.01; 0.37)*
	0.4 ± 1.5	2.5 ± 7.3	0.44 (0.10; 0.78)*

<sup>b</sup> incident ecstasy users vs persistent ecstasy-naives at follow-up (linear regression adjusted for baseline scores, substance use log-transformed)

\* significant difference, *p* < 0.05

the two groups were significantly different in terms of cannabis use (48.0 versus 17.6 joints in the last year,  $B = 0.69$ , 95% CI 0.01 to 1.37). In addition, between baseline and follow-up, ecstasy users reported a significantly higher increase in the use of alcohol ( $B = 0.21$ , 95% CI 0.02 to 0.39), cannabis ( $B = 0.60$ , 95% CI 0.09 to 1.10), amphetamines ( $B = 0.19$ , 95% CI 0.01 to 0.37), and cocaine ( $B = 0.44$ , 95% CI 0.10 to 0.78) and a significantly smaller increase in age ( $B = -0.28$ , 95% CI -0.46 to -0.09) and in years of education ( $B = -0.49$ , 95% CI -0.94 to -0.05) (probably because a shorter mean follow-up period in incident ecstasy users) compared to persistent ecstasy-naive subjects.

### **Predictive value of symptoms of depression, impulsivity, and sensation seeking on future ecstasy use**

Baseline total scores on the BDI, BIS, and SBL of all 64 incident ecstasy users and all 94 persistent ecstasy-naive subjects did not predict incident ecstasy use between baseline and follow-up (model 1, total scores, data not shown). Years of education and use of cannabis, alcohol, and cocaine correlated with future ecstasy use ( $p < 0.20$ ), so they were included in model 2 as potential confounders. Also after controlling for these potential confounders, we did not observe a significant predictive effect of the baseline total scores of BDI, BID or SBL on future ecstasy use (model 2, total scores). However, the model showed a positive predictive effect of last year cannabis use on future ecstasy use (OR = 1.23, 95% CI 1.03 to 1.46). The analyses of subscales (model 1, subscale scores) showed a positive predictive value of the experience seeking subscale score of the SBL on future ecstasy use (OR = 1.05, 95% CI 1.00 to 1.10) and an unexpected negative predictive value of the thrill and adventure seeking subscale score of the SBL on future first ecstasy use (OR = 0.95, 95% CI 0.91 to 1.00). After adjustment for potential confounders (model 2, subscale scores), only the negative predictive value of the thrill and adventure seeking subscale score on future first ecstasy use remained significant (OR = 0.95, 95% CI 0.91 to 1.00). Also using the subscale model, last year cannabis use had a positive predictive value on future ecstasy use (OR = 1.30, 95% CI 1.08 to 1.56).

### **Influence of ecstasy use on depression, impulsivity, and sensation seeking**

Table 2 shows the total and subscale scores on the BDI, BIS, and SBL of the groups of 59 incident ecstasy users and 61 persistent ecstasy-naive volunteers who completed the follow-up session. The linear regression analyses, with correction for baseline scores on depression, impulsivity, or sensation seeking (model 1), showed a significant effect of ecstasy use on the follow-up assessments of general sensation seeking ( $B = 0.54$ , 95% CI 0.20 to 0.87), SBL experience seeking ( $B = 1.76$ , 95% CI 0.09 to 3.42),

**Table 2:** Results from BDI, BIS and SBL self-report questionnaires.

	Baseline		Follow-up		Regression coefficient <i>B</i> (95% CI) model 1
	Persistent ecstasy naives ( <i>N</i> = 61)	Future ecstasy users ( <i>N</i> = 59)	Persistent ecstasy naives ( <i>N</i> = 61)	Incident ecstasy users ( <i>N</i> = 59)	
<b>Beck Depression Inventory</b>					
Total	3.6 ± 4.1	4.2 ± 3.8	3.4 ± 3.5	4.6 ± 4.9	0.86 (-0.47; 2.18)
<b>Barrat Impulsiveness Scale</b>					
Attentional	16.5 ± 3.5	17.1 ± 3.1	17.1 ± 3.3	18.2 ± 3.6	0.67 (-0.21; 1.56)
Motor	22.9 ± 4.2	22.8 ± 2.9	22.9 ± 4.3	23.1 ± 3.6	0.20 (-0.93; 1.33)
Non-planning	26.7 ± 4.8	26.7 ± 4.1	27.0 ± 4.9	27.7 ± 4.6	0.73 (-0.49; 1.96)
Total	68.0 ± 10.5	68.7 ± 7.1	68.9 ± 10.5	71.3 ± 9.8	1.71 (-0.69; 4.12)
<b>Sensation Seeking Scale (SBL)</b>					
Thrill and adventure seeking	46.3 ± 7.4	44.1 ± 7.2	45.3 ± 7.0	43.7 ± 8.0	0.22 (-1.42; 1.85)
Experience seeking	45.0 ± 8.0	47.1 ± 9.0	44.5 ± 7.9	48.0 ± 9.1	1.76 (0.09; 3.42)*
Boredom susceptibility	41.9 ± 6.6	41.3 ± 6.3	39.4 ± 7.0	40.6 ± 6.8	1.63 (-0.25; 3.51)
Disinhibition	39.8 ± 5.9	40.4 ± 5.8	38.6 ± 6.4	42.3 ± 5.6	3.31 (1.74; 4.88)*,†
General sensation seeking	13.6 ± 1.4	13.6 ± 1.5	13.2 ± 1.5	13.7 ± 1.5	0.54 (0.20; 0.87)*,†

\* significant difference between incident ecstasy users vs persistent ecstasy-naives at follow-up (model 1, linear regression adjusted for baseline scores)

† significant differences between incident ecstasy users vs persistent ecstasy-naives at follow-up adjusted for potential confounders (model 2, backward linear regression adjusted for baseline scores and potential confounders, substance use log-transformed, data not shown in table)

Values are uncorrected for covariates and expressed as mean ± SD, outcomes as regression coefficients (95% CI).

and SBL disinhibition ( $B = 3.31$ , 95% CI 1.74 to 4.88). After correction for potential confounders (model 2), the effect of ecstasy use on the SBL general score ( $B = 0.51$ , 95% CI 0.20 to 0.83) and the disinhibition subscale scores ( $B = 3.25$ , 95% CI 1.74 to 4.76) remained statistically significant. Within the group of incident ecstasy users we found no significant effects of the cumulative dose of ecstasy on the follow-up scores of the questionnaires (model 3).

## DISCUSSION

The main aim of the current study was to establish the direction of the relationship between ecstasy use and depression, impulsivity, and sensation seeking, because it is still unknown and debated whether subjects with higher levels of depression, impulsivity, and sensation seeking are predisposed to start using ecstasy or whether ecstasy use leads to higher levels of depression, impulsivity, and sensation seeking. To our knowledge, this is the first longitudinal study that prospectively examined the

relationship between ecstasy use and depression, impulsivity, and sensation seeking. Incident ecstasy users and persistent ecstasy-naive volunteers were recruited by the same procedures and were very similar at baseline on potential confounders, except for the use of cannabis which was significantly higher in the incident ecstasy users.

First, we hypothesized that higher levels of depression, impulsivity, and sensation seeking would predispose for future ecstasy use in ecstasy-naive young adults. However, in our study population we found no evidence that higher total scores on the depression, impulsivity, or sensation seeking questionnaires predict incident ecstasy use. Higher scores on the experience seeking subscale of the SBL did predict future ecstasy use, which could have been expected because this sub-scale reflects 'a search for new sensory and psychological experiences and a unconventional life-style'. However, this effect disappeared after adjustment for confounding. In contrast, even after correction for potential confounders we found that subjects with a lower baseline score on the thrill and adventure seeking subscale of the SBL had a higher risk for future ecstasy use. This subscale reflects 'the need for participation in sports and activities with a strong accent on speed and danger', so it is not surprising that this subscale does not predict future ecstasy use, although the negative predictive effect is unexpected and unaccountable. Our findings are at odds with the findings of other studies that suggested that depression, impulsivity, and sensation seeking are risk factors for substance use<sup>38-40</sup>. One possible explanation is that our study group, including the control group, is probably not representative for the general population of young adults, because at baseline we selected subjects with a relatively high risk for first time ecstasy use according to their intention to start using ecstasy in the near future and ecstasy use among their friends. Moreover, subjects were willing to take part in a rather challenging research project including brain scanning, neuropsychological examination, and blood sampling<sup>42</sup>. When we compare the baseline scores of our population ( $N = 158$ ) with 'normal' scores of volunteers of approximately the same age in other studies, our population has higher scores on sensation seeking and impulsivity: mean score on the general sensation seeking scale of  $13.5 \pm 1.5$  in our baseline population is somewhat higher than the general scores on the SBL of  $12.4 \pm 1.9$  and  $12.8 \pm 1.7$  for males, and  $11.7 \pm 2.0$  and  $12.4 \pm 1.7$  for females reported in two Dutch university student populations<sup>48,49</sup>. In addition, on the thrill and adventure seeking subscale our population scored higher with  $45.1 \pm 7.7$  compared to  $39.9 \pm 8.8$  and  $41.0 \pm 9.0$  for males, and  $37.8 \pm 9.6$  and  $37.7 \pm 9.9$  for females reported in the Dutch university student populations. Also the baseline total score on the BIS of  $66.3 \pm 8.8$  (we had to leave one item out for comparability with the English version that contains one item less than the Dutch version) of our population is slightly higher than the total scores of  $63.8 \pm 10.2$  in university undergraduates from the United States<sup>46</sup> and of  $64.1 \pm 10.1$  in Italian college undergraduates<sup>54</sup>. Compared to a sample of 192 university psychology students with a mean BDI score of  $6.5 \pm 7.3$ ,

the baseline depression scores of our population is lower with a mean of  $3.9 \pm 4.2$ <sup>55</sup>. However, these differences are limited for SBL to about one standard deviation, for BDI to about half a standard deviation, and for BIS to less than one third of a standard deviation. Therefore, generally high sensation seeking and impulsivity scores, and low depression scores in our study population are probably not the single explanation for the absence of predictive values of sensation seeking, impulsivity, and depression on future ecstasy use in our study population. Probably more important are the lower variances in outcomes in our population than in the general student population, appearing from lower standard deviations on SBL, BIS, and BDI. This is probably caused by the selection procedure and could have hampered the finding of positive predictive values.

An additional finding of our study was that future ecstasy use was predicted by the amount of cannabis use during last year prior to baseline. Although all subjects at baseline came from the same selected population with high-risk to start using ecstasy in near future it is not surprising and known from literature that subjects with more experience in other drugs are more likely to start using ecstasy as well<sup>56,57</sup>. The predictive effect of cannabis use on future first ecstasy use is likely to be even greater in a general population, because last year prevalence of cannabis use in our persistent ecstasy-naïve control group was 69% which is much higher than in the general population of the same age group<sup>58,59</sup>.

An important finding is the increase of general sensation seeking and of disinhibition between baseline and follow-up in incident ecstasy users relative to persistent ecstasy-naïve controls. However, table 2 suggests that these significant effects seem to result from a combination of a slight increase in sensation seeking in incident ecstasy users and a decrease in sensation seeking in the persistent ecstasy-naïve volunteers during the follow-up period. This difference between ecstasy users and non-users in general sensation seeking and disinhibition, reflecting behaviour that relieves social inhibition through activities such as party going, gambling, and sex, might be caused by a difference in serotonergic activity in the brain, which is believed to play an important role in sensation seeking behaviour<sup>9</sup>. It is known that sensation-seeking scores decline with age, after having reached a peak in late adolescence<sup>60</sup>. Therefore we could speculate that this normal maturation occurred in the persistent ecstasy-naïves, while this decrease in sensation seeking failed to occur or is delayed in incident ecstasy users. A possible explanation is that consumption of psychoactive agents might affect neurobiological and psycho-physiological strengths and weaknesses that predispose or protect an individual from psychoactive abuse, especially during adolescence<sup>61,62</sup> and possibly also during young adulthood. In line with this, the incident ecstasy users showed a relative increase in use of alcohol, cannabis, amphetamines, and cocaine and a relative 'decrease' in years of education between baseline and follow-up compared to the persistent ecstasy-naïves. An alternative explanation is that shared factors, such

as lifestyle or personality have 'caused' both increased sensation seeking, increased substance use and incident ecstasy use.

We did not find any effect of ecstasy use on depression and impulsivity. This is in contrast with results of previous studies that reported ecstasy users to have higher levels of depression and/or impulsivity<sup>15,17,19,21,22,27,28</sup>, although these studies did not have baseline measurements prior to first ecstasy use. The absence of a clear effect of ecstasy on mood and impulsivity might be related to the fact that in the present study most incident ecstasy users only experimented with ecstasy use on a single or a few occasions and almost no heavy users were involved. Therefore, it is likely that ecstasy-induced depression and impulsivity only becomes apparent after higher cumulative dosages. This would be in concordance with findings that depression<sup>63</sup>, impulsivity<sup>64</sup>, and especially memory problems in ecstasy users are dose-related<sup>65-68</sup>, although it was shown that even novice ecstasy users who used ecstasy less than 10 times reported a diversity of problems which they attributed to their ecstasy use<sup>63</sup>. Especially because there is growing interest in the possible medical benefits of low dose ecstasy as an adjuvant to psychotherapy<sup>69,70</sup>, it is an important finding that low doses of ecstasy use do not seem to cause increased depression or impulsivity.

In spite of the prospective nature this study has some limitations. First, although it is possible with the current prospective study design to establish the temporal direction of relationships between variables, it is still impossible to establish with certainty whether relationships are really causal in origin, especially because it is not possible to perform a perfect randomized study on ecstasy-neurotoxicity in humans. Therefore, causal interpretation of the observed effects is hampered because there might be residual confounding (due to imperfect measurement of some confounders and the absence of measures of other potential confounders). Second, it is very difficult to include a representative sample of 'potential' novice ecstasy users. We noticed for example that higher educated subjects were more likely to participate in our study than subjects with lower levels of education. Moreover, we already discussed that subjects took part in a rather challenging research project including brain scanning, neuropsychological examination, and blood sampling. This probably induced selection of subjects with a high motivation, which may restrict the generalization of our findings. Third, although the follow-up period was relatively short and we cannot know whether subjects will become heavy users after the follow-up period, it seems that most of the incident ecstasy users only took one or a few tablets and can be classified as experimenters rather than as abusers. It is likely that these experimenters or novice users have a different personality profile (e.g. lower levels of impulsivity and sensation seeking) than subjects who become abusers or heavy (polydrug) users<sup>23,63,71</sup>. Therefore the results of our ecstasy users can probably not be extrapolated to heavy ecstasy users. On the other hand, the results are probably more representative for ecstasy users in general, because only 20-30% of the ecstasy users consume ecstasy on a regular

basis<sup>72</sup>. Fourth, inherent to the non-experimental design, there was no control over dosage and purity of the taken ecstasy tablets. Recent surveys in the Netherlands, however, confirm that in 2003 and 2004 95% of the tablets sold as ecstasy contain MDMA as the major component<sup>73,74</sup>. Fifth, the environmental circumstances under which ecstasy was taken in the simultaneous use of other substances in our study was heterogeneous. As the study mainly involved low-dose and moderate ecstasy users, it was impossible to control for patterns of use, although there are indications that this may play a significant role in potential damage<sup>75</sup>. Frequency and amount of drug use were mainly assessed through self-report questionnaires, although abstinence of drug use before measurements were verified by urine analyses. As in other studies, most of the incident ecstasy users also used cannabis and some of them also used cocaine and amphetamine, although we were able to adequately control for these confounders. It is virtually impossible to include only 'pure' ecstasy users, because most of them are polydrug users. Schuster even found that in a group of merely novice ecstasy users, 97% of them had used cannabis and 59% had used cocaine<sup>76</sup>. Sixth, because of medical ethics we had to inform subjects about the potential negative consequences of ecstasy use at baseline and this could have had consequences for future ecstasy use and self-reported outcomes such as depression, impulsivity, and sensation seeking. Although this information might have led to limited use of ecstasy this is not very likely because still 41% of the baseline population started the use of ecstasy. Regarding the self-report questionnaires, the information might have prompted subjects to report the effects about which they were warned. On the other hand, the period between informed consent at baseline and follow-up measurements was 16.0 months on average for incident ecstasy users so it is not very likely that many subjects will have remembered the exact contents of the original warning at follow-up. Moreover, ecstasy users did not differ from persistent ecstasy-naïves at follow-up on depression and impulsivity (mentioned in the information brochure), but they did differ on sensation seeking, while this was not mentioned in the information brochure. Seventh, we only measured depression, impulsivity, and sensation seeking with self-report questionnaires and we did not perform behavioural measurements. Self-reported psychopathological abnormalities might not only reflect real abnormalities but might be biased by lack of insight, neurotic personality or suggestibility of the subject and behavioural measurements are probably less sensitive to these forms of bias.

In conclusion, we could not confirm that depression, impulsivity, or sensation seeking are predictors for first ecstasy use in a selected population of young adults with a relative high risk of future ecstasy use, although future ecstasy use was predicted by cannabis use. In addition, our data suggest that relatively low doses of ecstasy use do not cause increased levels of depression or impulsivity, although we found an effect of ecstasy use on certain aspects of sensation seeking. This latter finding

may indicate that ecstasy use (or some related factor) prevents a normal decrease in sensation seeking observed in persistent non-users.

## ACKNOWLEDGEMENTS

The NeXT study was supported by The Netherlands Organization for Health Research and Development, Program Addiction. Questionnaires on drug use were obtained by courtesy of the Addiction Research Institute of the University of Utrecht. The authors thank Ivo Bisschops for his help on collecting all data and Maarten Koeter for his advice on the statistical procedures.

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**PART**

**V**

**Summary, general discussion  
and conclusions**

CHAPTER  
**13**

# **Summary, General Discussion and Conclusions**



In this final chapter, a summary and general discussion is provided of the findings described in this thesis. The general discussion will also contain some implications and directions for future research in this field. The chapter will end with some conclusions.

## SUMMARY

Although its popularity has somewhat decreased in the last few years, ecstasy (MDMA) is still one of the most widely used illicit recreational drugs, especially used by young people <sup>1</sup>. Since in the late eighties studies have started to examine the effects of ecstasy on the brain, concern has risen about its potential neurotoxicity, especially to the axons of the serotonin (5-HT) cells of the brain. Most of this evidence has been derived from studies in animals, mainly in rats and non-human primates <sup>2</sup>. Also in humans, many studies have been performed in recent years that indicated that ecstasy use might be neurotoxic in humans too. Most of these studies showed differences between heavy ecstasy users and non-users. It was shown that heavy ecstasy users had lower serotonin transporter (SERT) densities, had higher levels of depression, were more impulsive and scored worse on neuropsychological memory tests than non-users <sup>3-6</sup>. Despite the increasing evidence that heavy ecstasy use is neurotoxic in humans many questions remained unanswered. Most of the evidence of neurotoxicity in humans has been derived from retrospective studies. Therefore, it remains possible that differences between ecstasy users and non-users were not caused by ecstasy, but were pre-existent and even predisposed people to start using ecstasy <sup>7</sup>. Moreover, there are many potential confounders like use of drugs other than ecstasy, gender, lifestyle, and SERT polymorphism that could influence the results of studies in ecstasy users <sup>8</sup>. Finally, it is unknown what the effects of low dose ecstasy use are <sup>2,9-12</sup>.

Studying the potential neurotoxic effects of ecstasy on the brain is relevant because many young people all over the world use this drug or will start experimenting with it. Well-conducted studies can probably help these young adults in making their own benefit-risk analysis and making their own choices. In addition, it can guide governments and social organizations in the development of their prevention messages. Moreover, in the last few years, interest in the potential beneficial effects of MDMA as an adjunct in psychotherapy to reduce anxiety, tension or agitation in patients with post-traumatic stress disorder or in last stage cancer, has increased and the first studies to evaluate these beneficial effects have already started <sup>13,14</sup>. In this regard, a careful and scientifically funded analysis should be made in order to decide whether potential benefits outweigh potential risks.

The aim of this thesis was to gain more insight in the effects of ecstasy use on the brain, especially regarding causality, course and clinical relevance while considering

the most important potential confounders. Most of the studies in this thesis were part of the Netherlands XTC Toxicity (NeXT) study or studies on which the NeXT study was based. Part of the objectives of the NeXT study was addressed in this thesis. The objectives of the NeXT study included:

1. To study the causality of ecstasy use in observed brain pathology in humans;
2. To study the long-term course of brain pathology and related clinical characteristics in ecstasy users;
3. To study the clinical relevance of observed brain pathology in ecstasy users;
4. To study the dose-response characteristics of ecstasy use in the causation of brain pathology;
5. To study vulnerability and protective factors in the causation of brain pathology among ecstasy users;
6. To study potential neurotoxic consequences of ecstasy use in relation to the use of other drugs;
7. To study the presence of functional or structural damage to neurotransmitter systems other than serotonin following ecstasy exposure.

A combination of neuroimaging techniques, psychopathology questionnaires and neuropsychological tests were used as parameters of neurotoxicity. Neuroimaging included [ $^{123}\text{I}$ ] $\beta$ -CIT single photon emission computed tomography (SPECT) measuring SERT densities; proton magnetic resonance spectroscopy ( $^1\text{H}$ -MRS) measuring neurometabolites <sup>15-17</sup>; diffusion tensor imaging (DTI) measuring apparent diffusion coefficient (ADC) and fractional anisotropy (FA) of the diffusional motion of water molecules in the brain as indicators of axonal integrity <sup>18,19</sup>; and perfusion weighted imaging (PWI) measuring regional relative cerebral blood volume (rrCBV) indicative of brain perfusion <sup>20,21</sup>. With these combined imaging techniques we measured both structural ( $^1\text{H}$ -MRS and DTI) and functional ([ $^{123}\text{I}$ ] $\beta$ -CIT SPECT and PWI) aspects of neurotoxicity. Psychopathological assessment included the Beck Depression Inventory (BDI <sup>22</sup>), the Barratt Impulsivity Scale (BIS <sup>23</sup>) and the Spanning Behoeftelijst (SBL <sup>24-26</sup>), self-report questionnaires measuring symptoms of depression, impulsivity and sensation seeking, respectively <sup>27</sup>. Neuropsychological tests mainly included tests on various aspects of memory.

## Part I: Introduction

In **chapter 1** a general introduction in the history, the effects and the potential neurotoxicity of ecstasy is given and the outline of the thesis is presented.

Most of the studies in this thesis are based on the NeXT study. In **chapter 2** a detailed outline of the objectives and methods of the NeXT study is provided. The

NeXT study is a combination of different approaches with three substudies: (1) a cross-sectional substudy among heavy ecstasy users and controls with variation in drug use, which should provide information about potential neurotoxic consequences of ecstasy in relation to other drugs, (2) a prospective cohort substudy in ecstasy-naive subjects with high risk for future ecstasy use, which should provide information on the causality and short-term course of ecstasy use and potential neurotoxicity, and (3) a retrospective cohort substudy in lifetime ecstasy users and matched controls of an existing epidemiological sample, which should provide information on the long-term course and outcome of ecstasy use in the general population. This chapter also gives an overview of the imaging techniques, psychopathology questionnaires, neurocognitive tests, and measurements of potential confounders that were used. The final aim of the NeXT study, which also includes studies not described in this thesis, is to come to conclusions that can be used in prevention messages, clinical decision-making, and the development of an (inter)national ecstasy policy.

## Part II: Use and validity of imaging techniques in ecstasy research

In part II we reviewed the existing literature on neuroimaging studies in human ecstasy users and further validated [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT, one of the most frequently applied imaging technique to study ecstasy neurotoxicity in humans.

In **chapter 3** we reviewed the results of previous imaging studies in ecstasy users, in particular studies that used SPECT and positron emission tomography (PET) to measure SERT densities and  $^1\text{H}$ -MRS to measure certain brain metabolites. Most of the PET and SPECT studies provided suggestive evidence that people who heavily use ecstasy are at risk of developing subcortical, and probably also cortical reductions in SERT densities. These effects seem to be dose-dependent and probably (partly) reversible. Moreover, females may be more vulnerable than males. From this review it seems that  $^1\text{H}$ -MRS is a less sensitive technique for studying ecstasy's neurotoxic potential. The studies in this review were all retrospective and mainly included heavy ecstasy users, so we suggested that future ecstasy studies should address the effects of low dose ecstasy use and that longitudinal studies in human ecstasy users are needed to draw more definite conclusions on the causal role of ecstasy use in the observed differences in neuroimaging parameters between ecstasy users and non-users.

In **chapter 4** we assessed the validity of [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT in detecting MDMA-induced neurotoxicity *in vivo* in rats using a newly developed high-resolution pinhole SPECT system. We showed that both *in vivo* and *ex vivo*, thalamic, but not striatal, uptake ratios were reduced after MDMA treatment. This suggests that [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT is able to detect MDMA-induced loss of SERTs and therefore may be a

promising technique to perform serial studies on MDMA-induced serotonergic neurotoxicity in living small animals.

Although [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT has already been used to assess SERT densities in the human brain, there was still discussion about its validity because [ $^{123}\text{I}$ ] $\beta$ -CIT does not bind selectively to SERTs but also to dopamine transporters (DATs). In **chapter 5** we aimed to investigate the validity of [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT to measure SERT densities in both SERT-rich and SERT-low areas in the living human brain using a double-blind, placebo-controlled, crossover design with the selective serotonin reuptake inhibitor (SSRI) citalopram. We report that citalopram reduced [ $^{123}\text{I}$ ] $\beta$ -CIT binding ratios in SERT-rich midbrain and (hypo)thalamus. Binding ratios were also lower after citalopram in SERT-low cortical areas, but statistical significance was only reached in several cortical areas using a voxel-by-voxel analysis and not with a region of interest (ROI) analysis. In addition, we showed that citalopram increased binding ratios in the DAT-rich striatum. The results show that [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT is a valid technique to study SERT binding *in vivo* in human brain in SERT-rich areas. Although some evidence was provided that [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT may be used to measure SERTs in SERT-low cortical areas, these measurements must be interpreted with caution.

The fact that [ $^{123}\text{I}$ ] $\beta$ -CIT does not selectively bind to SERTs but also to DATs is a disadvantage when one aims to study the serotonin system, like in ecstasy users. Therefore, a new radiotracer [ $^{123}\text{I}$ ]ADAM has been developed with a high affinity for SERTs but not for other transporters like DATs, which makes it possible to assess SERTs more selectively. In **chapter 6** we examined the optimal time course of [ $^{123}\text{I}$ ]ADAM binding to central SERTs in young adults. The time of peak-specific [ $^{123}\text{I}$ ]ADAM binding was highly variable among subjects, but specific binding in the SERT-rich (hypo)thalamus peaked within 5 h post injection (p.i.) in all subjects. Moreover, in this brain area, binding ratios of specific to nonspecific binding did not significantly change between 3 and 6 h p.i., and peaked 5 h p.i. Therefore, we suggest that 5 h p.i. is an optimal time point for single-scan [ $^{123}\text{I}$ ]ADAM SPECT studies in humans.

### Part III: Retrospective studies in heavy ecstasy users

There is an ongoing discussion whether previously reported neurotoxic effects are caused by ecstasy, by other drugs or by the combinations of drugs<sup>3,28-32</sup>. Therefore, in **chapter 7** we aimed to distinguish the specific/independent effects of ecstasy and the relative contributions of amphetamine, cocaine, and cannabis on the brain in a sample with variation in type and amount of drugs that were used with a combination of  $^1\text{H}$ -MRS, DTI, PWI and [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT. Heavy ecstasy use showed no effect on  $^1\text{H}$ -MRS brain metabolite ratios and DTI-derived ADC. However, heavy ecstasy use was associated with lower FA in the thalamus, higher rrCBV in the thalamus and temporal grey matter and lower [ $^{123}\text{I}$ ] $\beta$ -CIT binding in the thalamus, frontal

grey matter, and temporal grey matter. After adjusting for the use of drugs other than ecstasy and potential confounders (gender, verbal IQ, smoking) there was still a significant specific effect of ecstasy on the brain imaging parameters in the thalamus. Amphetamine and cocaine had a significant effect on some outcome parameters in brain areas other than the thalamus, but these findings were less consistent and converging than the robust findings associated with ecstasy use. Cannabis had no effect on any of the outcome parameters. This study therefore suggests strong converging evidence for a specific toxic effect of ecstasy on serotonergic axons in the thalamus with decreased [ $^{123}\text{I}$ ] $\beta$ -CIT binding, probably reflecting damage to terminals of serotonergic axons, with a related decrease in FA due to axonal loss and increased rrCBV due to vasodilatation caused by sustained serotonin depletion.

Because serotonin is important for many neurocognitive and psychopathological processes, like memory and mood<sup>33-36</sup> we also looked at potential clinical consequences of ecstasy use. In **chapter 8** we assessed the effects of ecstasy use on mood, measured with the BDI and the composite international diagnostic interview (CIDI) and focussed on its association with SERT densities, measured with [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT, dose, and gender. The prevalence of clinical depression assessed with CIDI did not differ between groups of moderate ecstasy users, heavy ecstasy users, former heavy ecstasy users, and drug-using but ecstasy-naïve controls. However, BDI scores were higher in former heavy ecstasy users than in ecstasy-naïve controls. Moreover, the total number of ecstasy tablets taken lifetime was associated with higher BDI scores for depressive mood. We did not find that depressed mood in ecstasy users was associated with gender or with a decrease in SERT density.

In **chapter 9**, the effect of moderate, heavy, and former ecstasy use on cognitive function was investigated. As females may be more vulnerable for the effects of ecstasy than males<sup>37-39</sup> and as SERTs are important in the regulation of synaptic serotonin transmission, we examined whether the effects of ecstasy on cognition, measured with neuropsychological tests, were different for females and males and for subjects with a different polymorphism in the serotonin transporter promoter gene region (5-HTTLPR). Heavy and former ecstasy users performed poorer on memory tasks than controls, while moderate ecstasy users did not. There was no difference between groups on reaction times or attention/ executive functioning. We also did not observe a significant effect of 5-HTTLPR or gender on test performance.

## Part IV: Prospective studies in low dose ecstasy users

In part IV we describe the prospective substudies of the NeXT study, in which for the first time sustained effects of ecstasy were prospectively assessed in novel users. For this purpose a group of 188 ecstasy-naïve subjects with an increased risk for future ecstasy user were assessed at baseline and followed during a period of

about 18 months. In **chapter 10**, the first 30 incident ecstasy users were assessed with a combination of  $^1\text{H-MRS}$ , DTI and PWI and self-report questionnaires on psychopathology before and quite soon after their first ecstasy use (mean of 1.8 ecstasy tablets). As brain metabolites and FA, parameters of structural neuronal damage, did not change after ecstasy use, we found no indications that incidental ecstasy use leads to extensive axonal damage. However, we did find sustained decreases in rrCBV in the thalamus, dorsolateral frontal cortex and superior parietal cortex, and a decrease in ADC in the thalamus after ecstasy use. This may indicate that even a low dose of ecstasy can induce prolonged vasoconstriction in some brain areas, although it is not known yet whether this effect is permanent. However, this has to be replicated in additional studies, because after correction for multiple comparisons only the rrCBV decrease in the dorsolateral frontal cortex remained significant. We also observed a small but significantly increase in impulsivity and small but significant decrease in depression scores after ecstasy use.

At the end of the follow-up period we assessed the effects of ecstasy use on the brain with SPECT and MR imaging parameters and psychopathology self-report questionnaires by comparing 59 incident ecstasy users (mean use of 6.0 tablets) with 56 persistent ecstasy-naive controls. Comparisons were corrected for baseline measurements. In **chapter 11** we describe that compared to persistent ecstasy-naive subjects, novel, mainly, low dose, ecstasy users showed a decreased FA, increased ADC and decreased rrCBV in certain brain areas, mainly the basal ganglia. This suggests sustained vasoconstriction and probably damage to axons of brain neurons due to low dosages of ecstasy. Although we did not observe changes in SERT densities and neurometabolites, these results suggest that ecstasy even in low doses may have sustained effects on the brain.

In **chapter 12** we assessed the relationship between ecstasy use and self-reported depression, impulsivity, and sensation seeking in (almost) the same prospective study group. We found that depression, impulsivity, and sensation seeking did not predict first time ecstasy use in this population of young adults with the intention to start using ecstasy. At the follow-up session, a significant effect of ecstasy use on the general and the disinhibition subscales of the sensation seeking scale were observed, while no effects of ecstasy use were found on depression and impulsivity scores. This suggest that low level ecstasy use does not seem to cause depression or impulsivity, although low level ecstasy use may increase (certain aspects of) sensation seeking.

## GENERAL DISCUSSION

The aim of the studies described in this thesis was to increase knowledge concerning yet unanswered questions about the causality, course, and clinical relevance of the potential neurotoxicity of ecstasy in humans and to assess the role of dose-response characteristics, vulnerability and protective factors, and the relation between ecstasy use and the use of other drugs in the potential neurotoxicity of ecstasy (**chapter 2**). In addition, the validity of [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT in the detection of ecstasy-induced decreased in SERT was evaluated. In the following general discussion the major findings are addressed in relation to the main research questions.

### Causality

The issue of causality of ecstasy use in the observed differences between ecstasy users and non-users is very complex due to the use of cross-sectional and retrospective designs with lack of baseline data and inadequate control of potential confounders<sup>8,32</sup>. In this thesis three prospective studies were described in **chapters 10, 11, and 12**, which are the first prospective studies focussing on the sustained effects (after abstinence of at least two weeks) of ecstasy in novel ecstasy users by comparing parameters before and after first ecstasy use. Results from both brain imaging studies (**chapters 10 and 11**) showed no changes in SERT densities ([ $^{123}\text{I}$ ] $\beta$ -CIT SPECT) and brain metabolites ( $^1\text{H}$ -MRS) after the use of low doses of ecstasy. However, both studies did show a decrease in rrCBV values, suggesting sustained vasoconstriction even after low dose ecstasy use, although the brain areas with significantly decreased rrCBV values differed between both studies. More difficult to interpret are the DTI results, because FA in the centrum semiovale was increased in the intermediate follow-up session (**chapter 10**) and decreased in the final follow-up session (**chapter 11**). Moreover, in the final follow-up session FA was decreased in the thalamus and increased in the globus pallidus. Although we hypothesized that the increased FA in the globus pallidus might be related to the significantly decreased rrCBV in this area and the decreased FA may indicate axonal loss in this area, these findings should be interpreted with caution. Also the ADC in the thalamus showed a discrepancy between follow-up sessions, being significantly decreased at the intermediate follow-up and increased at the final follow-up compared to baseline, although this latter was only a relative increase compared to persistent ecstasy-naïves. In the same prospective study group, the intermediate follow-up suggested a small but significantly increased impulsivity and decreased depression after ecstasy (**chapter 10**), although this was not confirmed in the final follow-up when compared to persistent ecstasy-naïves (**chapter 12**). On the other hand, the final follow-up session suggested that ecstasy might increase certain aspects of sensation seeking.

The advantage of the prospective design is of course the possibility to compare the same parameters in the same subjects before and after the first use of ecstasy. Moreover, incident ecstasy users and persistent ecstasy-naïve volunteers were recruited by the same procedures and were very similar at baseline on potential confounders. In addition, the (potential confounding) use of amphetamines and cocaine in the prospective population was very low. Therefore, it is very likely that the observed changes in brain perfusion and potentially also in diffusion and psychopathology are actually caused by ecstasy use. On the other hand, despite the prospective design, it is still impossible to establish with certainty whether relationships are really causal in origin, especially because it is not possible to perform a perfect randomized study on ecstasy-induced neurotoxicity in humans. Therefore, residual confounding may still be responsible for (part of) the observed differences, hampering the causal interpretation.

Disadvantage of the prospective design is that most subjects only experimented with the use of ecstasy a single or a few times during the follow-up period, so the average cumulative dose was small with an average dose of only 1.8 (**chapter 10**) and 6.0 tablets (**chapters 11 and 12**) and only few subjects used more than 10 ecstasy tablets. Therefore, we assessed the effects of heavy ecstasy use using a retrospective study design and using the same imaging parameters. Although it is not possible from a retrospective study to establish causality, a strong relationship was suggested between ecstasy use and converging changes in brain parameters in the thalamus in **chapter 7**. By using linear regression analyses in a sample with variations in amount and drug used it was possible to minimize correlations between different drugs and to distinguish between the effects of ecstasy and other drugs to a certain extent. Also in the retrospective study of **chapter 8**, causality between ecstasy use and increased depressive symptoms was suggested because of a significant correlation between cumulative ecstasy dose and higher BDI scores.

## Course

Another main research question was to study the long-term course of ecstasy-induced neurotoxicity and related clinical characteristics. **Chapter 8** showed higher depression scores measured with BDI in former heavy ecstasy users who were abstinent for at least 1 year, although this was not associated with decreases in SERT densities. A previous study in the same study population showed significantly decreased SERT densities only in female current heavy ecstasy users and not in males or in former heavy ecstasy users<sup>39</sup>, suggesting that SERT densities seem to recover, finding that was recently confirmed by others<sup>37</sup>. However, as shown in **chapter 9** and in a previous publication in this cohort<sup>40</sup>, both male and female former heavy ecstasy users performed worse on a verbal memory task than non-users. It may therefore be possible that, although SERTs seem to recover, the functionality of these transporters

is not optimal, for example by abnormal sprouting of the remaining serotonergic neurons or by upregulation of SERTs in the remaining serotonergic axons <sup>41</sup>.

Because the follow-up period in the prospective cohort was limited to about 18 months we cannot make firm conclusions regarding the long-term outcome of ecstasy use in this cohort. Answering this question in the prospective cohort would require future monitoring of this cohort. The retrospective cohort substudy of the NeXT study, in lifetime ecstasy users and matched controls of an existing epidemiological sample (**chapter 2**), will provide information on long-term course and outcome of ecstasy use in the general population, but this substudy is not described in this thesis.

## Clinical relevance

The clinical consequences of the decreased rrCBV values and probably changed diffusion values after low dose ecstasy use are not clear yet, especially because it is not known whether the effects are sustained but reversible or permanent. Moreover, as discussed above, the effects of low dose ecstasy on psychopathology seemed to be quite subtle (**chapters 10 and 12**). Also functional MRI (fMRI) in the same cohort showed no firm evidence for sustained effects of a low dose of ecstasy on working memory, selective attention or associative memory, neither at the behavioural nor at the neurophysiological level (not presented in this thesis) <sup>42</sup>. On the other hand, a neuropsychological study in the same prospective cohort showed that immediate and delayed verbal recall and verbal recognition was relatively decreased in incident ecstasy users (not presented in this thesis) <sup>43</sup>.

Clinical consequences may become more pronounced after heavy ecstasy use, as was confirmed in **chapters 8 and 9**, showing increased depressive symptoms measured with BDI in former heavy ecstasy users and poorer verbal memory performance in heavy and former heavy ecstasy users compared to non-users. On the other hand, no effect of ecstasy was observed on clinical depression as measured with CIDI or on measurements of reaction times, attention or executive functioning. The clinical relevance of the specific ecstasy effects on imaging parameters in the thalamus that were observed in **chapter 7** are not clear yet. However, fMRI findings in the same population suggested that ecstasy use was associated with reduced performance and altered brain activity for associative memory, although the effect of amphetamine on associative memory performance was much stronger than the effect of ecstasy. Ecstasy and other drug use had little effect on working memory and attention level (not presented in this thesis) <sup>44</sup>. Neuropsychological assessment in the same group suggested a specific sustained negative effect of ecstasy use on verbal memory, although the clinical relevance is not immediately clear, because test performance generally remained within normal range (not presented in this thesis) <sup>45</sup>. As all ecstasy

users in this study population did use ecstasy in the past 6 months, it is not clear yet whether these effects are permanent.

## Dose-response characteristics

The range of the cumulative ecstasy doses was probably too small in the prospective studies (almost exclusively low dose users) of **chapters 10 to 12** as well as in the heavy ecstasy study (almost exclusively heavy ecstasy users) of **chapter 7** to detect dose-response effects in the separate studies. However, there are differences in the outcomes between both study populations that may be explained by the difference in cumulative ecstasy doses, especially because exactly the same imaging techniques were used in both cohorts. While no changes in SERT densities were observed in the low dose users as measured with [<sup>123</sup>I]β-CIT SPECT (**chapter 11**), we did find decreased SERT densities in the thalamus of heavy ecstasy users (**chapter 7**), so the decreased SERT densities seem to be dose-dependent. This was also shown in previous studies<sup>39,46</sup>. A decrease in FA in the thalamus was observed in low dose ecstasy users (**chapter 11**) as well as in heavy ecstasy users (**chapter 7**). The dose-response relationship between ecstasy use and rrCBV values is more difficult to interpret because the low dose studies mainly showed decreased brain perfusion (**chapters 10 and 11**), while the heavy ecstasy study showed increased brain perfusion in some brain areas (**chapter 7**). Previously, a relationship between rrCBV values and time since last ecstasy tablets was suggested<sup>47-49</sup>, although we could not confirm this in our studies. Therefore, these apparent inconsistencies suggest there is a complex, yet to be unravelled, relationship between ecstasy use and serotonergic-mediated brain perfusion. This probably related to time since last tablet, cumulative dose, and adaptation of serotonergic transporters and receptors to the ecstasy-induced increase of serotonin in the (sub)acute stage and serotonergic depletion on the long-term.

We observed a dose-response relationship between cumulative ecstasy dose and increased depressive symptoms as measured with BDI (**chapter 8**). Although the intermediate follow-up session in the prospective study also showed a small but significantly increased BDI scores after low dose ecstasy use (**chapter 10**), this was not confirmed in the final follow-up session (**chapter 12**). In **chapter 9** a dose-response relationship was suggested between verbal memory and ecstasy dose, as heavy and former heavy ecstasy users performed worse on a verbal memory task, while moderate ecstasy users did not.

## Vulnerability and protective factors

Previous studies indicated that women may be more vulnerable to the effects of ecstasy than men<sup>37-39</sup> (see also the review of **chapter 3**). However, we did not

observe a clear effect of gender on any of the outcome parameters in the present studies. We also did not find a vulnerability effect of the short allele variant of the serotonin transporter promotor gene region (5-HTTLPR) on the effects of ecstasy on cognition (**chapter 9**), although a previously suggested a vulnerability effect of the short 5-HTTLPR variant on abnormal emotional processing in ecstasy users<sup>50</sup>. We also could not confirm that higher depression, impulsivity or sensation seeking would predict future first time ecstasy use (**chapter 12**).

## The role of other drug use in the potential neurotoxicity of ecstasy use

One of the main problems of ecstasy research is that the pure ecstasy user does barely exist<sup>51-53</sup>. Therefore, potential confounding effects of other drugs besides ecstasy should always be taken into account, even though ecstasy is the only drug with a selective serotonergic effect. The specific research question regarding the relationship between ecstasy use and use of other drugs in the potential neurotoxicity of ecstasy was addressed in **chapter 7**. We found a significant and specific effect of heavy ecstasy use on various brain imaging parameters in the thalamus, independent of other drugs. In the same study we did find that amphetamine and cocaine had significant effects on some outcome parameters in brain areas other than the thalamus, but these findings were less consistent and converging than the findings associated with ecstasy use. Cannabis had no effect on any of the outcome parameters.

Advantage of the prospective studies (**chapters 10 to 12**) was that subjects did hardly use hard drugs besides ecstasy and that until now there is no hard evidence for sustained effects of cannabis use on the brain, especially not in the moderate dosages that were used by our subjects.

## Methodology, assessments and confounders

In **chapter 2** it was discussed that the main research questions on causality, course and clinical relevance of the potential neurotoxicity of ecstasy did arise from the difficulty to overcome various methodological problems inherent to naturalistic human studies in this field of research. This includes inadequate sampling of users and controls, small samples, lack of drug-use analysis, restricted dose ranges, short follow up periods, and the use of cross-sectional and retrospective designs with lack of baseline data and inadequate control for potential confounders<sup>8,32,54-56</sup>. The strength and limitations of the studies described in this thesis were already discussed extensively in each chapter and therefore only a brief summary and discussion is provided here.

Clearly, one of the strengths of the studies described in this thesis are their study designs. First, the use of [<sup>123</sup>I]β-CIT SPECT to assess SERT densities in ecstasy users

was validated and reviewed extensively (**chapters 3 to 5**). In the cross-sectional sub-study in heavy ecstasy users, we applied a new approach to deal with confounding by polydrug use (**chapter 7**). The most innovative design was applied in the prospective sub-studies of **chapters 10 to 12** and, as far as we know, the NeXT project is the first and still the only that succeeded in assessing novice ecstasy users before and after first ecstasy use at such a large scale. Another strength of the current study is that we combined different imaging techniques, psychopathology questionnaires and neuropsychological assessments to assess different aspects of potential neurotoxicity. Besides the assessments presented in this thesis also neuropsychological and fMRI assessments were performed in the same samples of the heavy ecstasy sub-study and the prospective sub-study of the NeXT study <sup>42-45</sup>.

Limitations of the studies presented in this thesis are related to the lack of control on dose and purity of the ecstasy tablets taken, subject selection, potential confounding of poly-drug use, the imaging techniques, and correction for multiple comparisons.

Inherent to the naturalistic approach of our studies, there is uncertainty about variations in dosage and purity of ecstasy tablets taken. However, pill analysis confirms that in the Netherlands more than 95% of the tablets sold as ecstasy contain MDMA as the only (91.2%) or major (4.2%) component <sup>57-60</sup>. The mean concentration of MDMA in an ecstasy tablet was 78 mg in 2003 in the Netherlands <sup>59</sup>. Also the environmental circumstances under which ecstasy was taken and the simultaneous use of other substances were heterogeneous. Frequency and amount of drug use were mainly assessed through self-report questionnaires. Abstinence of drug use before assessments were verified by urine analyses, although sensitivity of these tests is limited as for most of the drugs the urine test will only be positive when drugs were taken in the last few days.

Also inherent to this field of research, subjects were selected and not randomly assigned, which may restrict the generalization of our findings. Subjects took part in a rather challenging research project including brain scanning, neuropsychological examination, and blood sampling. This probably induced selection of subjects with a high motivation. We also noticed that relatively higher educated subjects were more likely to participate in our studies than subjects with lower levels of education. Because of our selection strategy of the heavy ecstasy NeXT sub-study (**chapter 7**) we included selective ecstasy users and polydrug controls without experience with ecstasy use that are atypical and might be unrepresentative. In addition, because subjects were not randomly assigned in the different studies we cannot exclude some residual confounding of differences between ecstasy users and non-users on some outcomes, other than ecstasy use. Although we tried to limit the potential confounding effects by using unique study designs and adequate statistical models, we cannot totally exclude confounding by the use of other substances, such as alcohol, nicotine, cannabis, amphetamines and cocaine.

Because few studies used  $^1\text{H-MRS}$ , DTI and PWI to study neuronal damage in ecstasy users, little is known about the sensitivity and specificity of these techniques to detect ecstasy-induced neuronal damage, especially not for the detection of relatively small changes after low dose ecstasy use. Also, little is known about the reproducibility of our MR and SPECT measurements, especially not after a relatively long follow-up period of 18 months. Although other studies suggested that  $^1\text{H-MRS}$ , DTI and PWI are sensitive tools in various neuropsychiatric disorders<sup>4,61-69</sup> and that reproducibility is good<sup>70-73</sup>, additional (animal) studies are needed.

In most of our studies we did not use corrections for multiple comparisons, although we used multiple techniques as indicators for ecstasy-induced brain damage and multiple regions of interests. This increases probability of type I errors (false positive results). When Bonferroni corrections would be applied many of the significant findings would not remain significant, as was shown in **chapter 10**. On the other hand, the Bonferroni correction is probably too conservative especially in the prospective studies, as *a priori* we expected small effects because we studied early indicators of potential brain damage in subjects with only *low* cumulative doses of ecstasy use. Moreover, all imaging techniques and ROIs were chosen based on *a priori* hypotheses. Therefore, it is likely that Bonferroni correction would induce type II errors (false negative findings). The risk of such corrections was previously discussed by Rothman, who showed that correction for multiple comparisons can obscure possibly important findings<sup>74</sup>. Because of its social impact, additional research is needed to establish whether our current uncorrected significant findings can be replicated.

## Implications

The main objective of the NeXT study was to come to scientifically sound conclusions regarding the neurotoxicity of ecstasy that can be validly used in prevention messages, clinical decision-making, and the development of a (inter)national ecstasy policy. Although this thesis is only part of the NeXT study and the findings have to be interpreted together with the other substudies before final conclusions can be drawn and recommendations can be put forward, we want to briefly discuss the potential implications of the findings of this thesis.

The findings of the heavy ecstasy studies, showing strong converging evidence for a specific toxic effect of ecstasy on serotonergic axons in the thalamus (**chapter 7**), increased symptoms of depression (**chapter 8**), and poorer verbal memory performance (**chapter 9**) corroborate the general concern about the negative consequences of heavy ecstasy use on the brain and its related functional impairments. Therefore, we suggest public health measures should be taken to prevent heavy recreational use of ecstasy. For low dose incidental ecstasy use this is less clear, because although we

found indications of sustained decreased perfusion in some brain areas, there was no consistent evidence for structural brain damage or clinically relevant consequences (**chapters 10, 11, and 12**). Other studies also showed that adverse effects of a low ecstasy dose are rather limited <sup>75,76</sup>. On the other hand, the observed decreased rrCBV does suggest prolonged vasoconstriction and decreased FA probably axonal loss even in low dose ecstasy users. In addition, neuropsychological data from the same study population suggest that even low dose ecstasy use is associated with small but significantly decreases in verbal memory relative to non-users <sup>43</sup>. Although we do not know whether these effects are permanent, we cannot conclude that incidental ecstasy use safe for the human brain. Moreover, there are various factors (e.g. poor metabolism, hypertension, young age, simultaneous use of other substances, environmental conditions) that might contribute to individual or situational vulnerability for acute adverse effects and long-term neurotoxicity of ecstasy <sup>77-80</sup>. Therefore, we think that current users and potential future users should be informed about the potential risks of even incidental ecstasy use. Regarding the prescription of MDMA as adjuvant in psychotherapy a well-considered benefit-risk analysis should be made and more research on this topic is necessary.

## Future directions

The first important future step is to finish all substudies of the NeXT project, including the neuropsychology and fMRI assessments in the heavy ecstasy and the prospective sub-studies and the retrospective cohort sub-study, not presented in this thesis. An integration of these studies should be made to come to final conclusions and recommendations of this ambitious project.

Second, we are the first reporting on sustained effects of ecstasy in new low dose ecstasy users using a prospective design, so it is important that our findings will be replicated, preferably also in large-scale prospective studies. In line with this, more studies are needed on the acute and long-term effects of incidental or low to medium dose ecstasy use, instead of repeating studies in heavy ecstasy users, especially because most young adults will only experiment with ecstasy and only 20-30% will become regular users <sup>57</sup>.

Third, in most of the presented studies we do not know whether effects are permanent, because subjects were recent ecstasy users (within the last six months). Also relatively few other studies assessed the long-term effects of ecstasy. Therefore, it is important to gain more knowledge about the long-term effects of ecstasy in humans in future research. In this regard, it will be very interesting to monitor our current prospective cohort. This cohort creates a unique opportunity to re-examine the causality question, because of the expected increase in variation in dose, frequency and duration of ecstasy use within this group of novice users. One could

also hypothesize that there may be an additive effect of ecstasy use on ageing of the brain, i.e. that ecstasy use reinforces the normal age-related decrease in serotonin cells. This may implicate that effects of ecstasy on, for example, memory, would become only clinically relevant at older age when reserve capacity of the brain has declined. Therefore, future ecstasy research should also examine the effects in older (former) ecstasy users.

Fourth, future studies should continue to combine different neuroimaging techniques and neuropsychological and psychiatric assessments to facilitate convergence of evidence. Moreover, they should be designed to minimize potential confounding of lifestyle, demographics, polydrug use, and pre-existing psychiatric morbidity and cognitive dysfunctioning. Naturally, a detailed description of drug history should be collected and ideally this should be confirmed with urine and hair analysis. When assessing sustained effects of ecstasy, one should take into account a minimum duration of abstinence prior to assessments of at least 10 days, to avoid acute pharmacological effects.

Fifth, as the field of neuroimaging is quickly developing, a lot of technical improvements have been put through since the start of the NeXT project in 2001. While we used a MRI scanner with a 1.5T magnetic field in our studies, in 2007 3T is state of the art in brain research and the expectation is that an increasing amount of 7T scanners will be implemented for human brain research in the next few years. Theoretically, the sensitivity of the different MRI techniques to detect minor signs of neurotoxicity will increase with higher field MRI scanners, but this has to be confirmed in future studies. While our current results and the results of others suggest a limited sensitivity of  $^1\text{H}$ -MRS in detecting neurometabolite changes, at least in low to moderate ecstasy users, the results of high field  $^1\text{H}$ -MRS in other fields of research are promising. Also the value of more advanced techniques such as DTI fiber tracking have to be evaluated for their value in ecstasy research. For assessing brain perfusion, arterial spin labelling will become an important technique because with this technique it is possible to measure brain perfusion in quantitative instead of relative parameters and without the need for a contrast agent. Further, it is very likely that in future it will be possible to image serotonergic function in a more direct way using pharmacologic or molecular MRI techniques<sup>81</sup>. Regarding SERT SPECT imaging, more selective SERT ligands have been developed, such as [ $^{123}\text{I}$ ]ADAM (**chapter 6**)<sup>82</sup>. In future studies it has to be assessed whether these selective ligands will improve sensitivity to detect ecstasy-induced decreased SERT densities, especially in the SRT-low cortical areas. Although these improved techniques may improve sensitivity, one has to bear in mind that inter- and intra-individual differences will persist.

Finally, clinical trials with MDMA in patients (despite the ongoing and fierce debate on whether it is ethically justified and/or safe to do so) can also be of value in extending our knowledge on the effects of controlled and low dose ecstasy use on the

human brain and whether potential benefits of MDMA as adjuvant in psychotherapy may outweigh the potential risks.

## CONCLUSIONS

In conclusion, in this thesis we describe retrospective and prospective studies that assessed potential neurotoxic effects of the popular recreational drug ecstasy using neuroimaging, psychopathology questionnaires and neuropsychological assessments in different groups of ecstasy users. The research questions were especially aimed at increasing knowledge about causality, course, and clinical relevance of potential ecstasy-related (serotonergic) neurotoxicity in humans. Moreover, we studied the validity of [<sup>123</sup>I]β-CIT SPECT in assessing serotonin transporter densities. The results showed that [<sup>123</sup>I]β-CIT is indeed a valid technique to assess SERT densities, especially in SERT-rich brain areas and to some extent also in SERT-low cortical areas. We also found that heavy ecstasy use has a specific effect on the thalamus independent of the use of other drugs and that heavy ecstasy use is probably related to increased symptoms of depression and decreased verbal memory. Finally, the results suggest a causal relationship between low dose ecstasy use and sustained changes in brain perfusion, although no strong evidence was found that low ecstasy use does lead to axonal damage. Clinically, low dose ecstasy used does not seem to cause depression or impulsivity, but it may increase (certain) aspects of sensation seeking. Findings in low dose ecstasy users should be confirmed in future research.

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CHAPTER

14

# **Samenvatting, Algemene Discussie en Conclusies**



In dit laatste hoofdstuk wordt een samenvatting en algemene discussie gegeven van de bevindingen die in dit proefschrift worden beschreven. In de algemene discussie bespreken we ook enkele implicaties van onze bevindingen en aanbevelingen voor toekomstig onderzoek. Het hoofdstuk zal eindigen met de belangrijkste conclusies van dit proefschrift.

## SAMENVATTING

Hoewel de populariteit in de laatste jaren iets is afgenomen, is ecstasy (MDMA) nog één van de meest gebruikte illegale recreatieve drugs, vooral bij jongeren <sup>1</sup>. Sinds in de eind jaren '80 de eerste studies de gevolgen van ecstasy gebruik voor de hersenen onderzochten, is de bezorgdheid over de mogelijk neurotoxiciteit van ecstasy, vooral voor de axonen van de serotonine (5-HT) cellen van de hersenen, toegenomen. Neurotoxiciteit werd met name aangetoond in onderzoek met dieren, hoofdzakelijk bij ratten en primaten <sup>2</sup>. Ook bij mensen zijn de laatste jaren vele studies uitgevoerd die erop wijzen dat ecstasy gebruik ook bij mensen neurotoxisch zou kunnen zijn. De meeste van deze studies toonden verschillen aan tussen forse ecstasy gebruikers en niet-gebruikers. Men toonde aan dat forse ecstasy gebruikers een lagere serotonine transporter (SERT) dichtheid hadden, depressiever waren, impulsiever waren en slechter scoorden op neuropsychologische geheugentesten dan niet-gebruikers <sup>3-6</sup>. Ondanks het toenemende bewijs dat fors ecstasy gebruik neurotoxisch is voor de mens blijven vele vragen onbeantwoord. Vrijwel al het bewijs voor neurotoxiciteit is afkomstig van retrospectieve studies. Hierdoor is het mogelijk dat verschillen tussen ecstasy gebruikers en niet-gebruikers niet door ecstasy worden veroorzaakt, maar preëxistent waren of zelfs predisponeerden om met ecstasy gebruik te gaan beginnen <sup>7</sup>. Verder zijn er vele potentiële confounders, zoals gebruik van andere drugs dan ecstasy, geslacht, levensstijl, en het SERT polymorfisme, die de resultaten van studies bij ecstasy gebruikers kunnen beïnvloeden <sup>8</sup>. Tot slot is het onbekend wat de gevolgen van een lage dosering ecstasy gebruik zijn <sup>2,9-12</sup>.

Het onderzoeken naar de potentiële neurotoxische gevolgen van ecstasy voor de hersenen is relevant, omdat wereldwijd veel jongeren deze drug gebruiken of er mee zullen gaan experimenteren. Goed opgezette en uitgevoerde studies kunnen deze jongeren mogelijk helpen bij het maken van hun eigen risico calculatie en het maken van weloverwogen keuzes. Bovendien kan het overheden en sociale organisaties begeleiden bij de ontwikkeling van hun preventie beleid. Daarnaast is er in de afgelopen jaren toenemende belangstelling geweest voor de potentieel gunstige effecten van MDMA als aanvullend medicijn bij psychotherapie, om angst, spanning of agitatie te verminderen bij patiënten met een posttraumatische stress stoornis of in het terminale stadium van kanker <sup>13,14</sup>. Dit zou door middel van zorgvuldig

wetenschappelijk onderzoek geanalyseerd moeten worden, zodat duidelijk wordt of de potentiële voordelen groter zijn dan de potentiële risico's.

Het doel van de studies in dit proefschrift was om meer inzicht te verwerven in de gevolgen van ecstasy gebruik voor de hersenen, vooral wat betreft causaliteit, beloop en klinische relevantie, met inachtneming van de belangrijkste potentiële confounders. De meeste studies in dit proefschrift maakten deel uit van 'the Netherlands XTC Toxicity' (NeXT) studie of zijn studies waarop de NeXT studie is gebaseerd. Een deel van de doelstellingen van de NeXT studie werden in dit proefschrift behandeld. De doelstellingen van de NeXT studie omvatten:

1. Het onderzoeken van de causaliteit tussen ecstasy gebruik en geobserveerde hersenpathologie bij mensen;
2. Het onderzoeken van het lange termijn beloop van deze hersenpathologie en gerelateerde klinische consequenties bij ecstasy gebruikers;
3. Het onderzoeken van de klinische relevantie van geobserveerde hersenpathologie bij ecstasy gebruikers;
4. Het onderzoeken van dosis-respons kenmerken in het veroorzaken van hersenpathologie bij ecstasy gebruikers;
5. Het onderzoeken van risico factoren en beschermende factoren bij het ontstaan van hersenpathologie bij ecstasy gebruikers;
6. Het onderzoeken van potentiële neurotoxische gevolgen van ecstasy gebruik in relatie tot het gebruik van andere drugs;
7. Het onderzoeken van mogelijke functionele of structurele schade aan andere neurotransmittersystemen dan het serotonine systeem door ecstasy gebruik.

Neurotoxiciteit werd onderzocht met behulp van een combinatie van neuroimaging technieken, psychopathologie vragenlijsten en neuropsychologische tests. Neuroimaging bestond uit [ $^{123}\text{I}$ ] $\beta$ -CIT single photon emissie computer tomografie (SPECT), dat de dichtheid van de SERT meet; proton magnetische resonantie spectroscopie ( $^1\text{H}$ -MRS), dat neurometabolieten meet<sup>15-17</sup>; diffusie tensor imaging (DTI) dat de apparent diffusie coëfficiënt (ADC) en fractionele anisotropie (FA) meet van de diffusie van water moleculen in de hersenen als indicatoren van de integriteit van de axonen<sup>18,19</sup>; en perfusie gewogen imaging (PWI), dat het regionale relatieve cerebrale bloed volume (rrCBV) meet als indicatie van hersenperfusie<sup>20,21</sup>. Met deze gecombineerde imaging technieken was het mogelijk zowel structurele ( $^1\text{H}$ -MRS en DTI) en functionele ([ $^{123}\text{I}$ ] $\beta$ -CIT SPECT en PWI) aspecten van neurotoxiciteit te bepalen. Psychopathologisch onderzoek bestond uit de Beck Depression Inventory (BDI<sup>22</sup>), de Barratt Impulsivity Scale (BIS<sup>23</sup>) en de Spannings Behoeftelijst (SBL<sup>24-26</sup>), zelfrapportage vragenlijsten, die respectievelijk symptomen van depressie, impulsiviteit en spanningsbehoefte ('sensation seeking') meten<sup>27</sup>. Met

het neuropsychologisch onderzoek werden met name verschillende aspecten van het geheugen getest.

## Deel I: Inleiding

In **hoofdstuk 1** wordt een algemene inleiding gegeven in de geschiedenis, de effecten en de potentiële neurotoxiciteit van ecstasy en daarnaast wordt een overzicht gegeven van de hoofdstukken in het proefschrift.

De meeste studies in dit proefschrift zijn gebaseerd op de NeXT studie. In **hoofdstuk 2** wordt een gedetailleerd overzicht van de doelstellingen en de methodes van de NeXT studie gegeven. De NeXT studie is een combinatie van verschillende benaderingen met drie substudies: (1) een cross-sectionele substudie onder forse ecstasy gebruikers en controles, met variatie in hun drugs gebruik, die informatie zou moeten verschaffen over potentiële neurotoxische gevolgen van ecstasy in relatie tot andere drugs, (2) een prospectieve cohort substudie bij ecstasy-naïeve vrijwilligers met een hoog risico op toekomstig ecstasy gebruik, die informatie zou moeten verschaffen over de causaliteit en het korte termijn beloop van ecstasy gebruik en potentiële neurotoxiciteit, en (3) een retrospectieve cohort substudie bij lifetime ecstasy gebruikers en gematchte controles van een bestaand epidemiologisch sample, die informatie zou moeten verschaffen over het beloop en de lange termijn gevolgen van ecstasy gebruik in de algemene populatie. Dit hoofdstuk geeft ook een overzicht van de gebruikte imaging technieken, psychopathologie vragenlijsten, neuropsychologische tests en potentiële confounders. Het uiteindelijke doel van de NeXT studie, die ook studies bevat die niet beschreven worden in dit proefschrift, is om tot wetenschappelijke conclusies te komen die gebruikt kunnen worden voor preventiedoeleinden, klinische besluitvorming en de ontwikkeling van een (inter)nationaal ecstasy beleid.

## Deel II: Gebruik en validiteit van imaging technieken bij ecstasy onderzoek

In deel II hebben we de bestaande literatuur over neuroimaging studies bij menselijke ecstasy gebruikers gereviewed en [<sup>123</sup>I]β-CIT SPECT, één van de meest toegepaste imaging technieken om ecstasy neurotoxiciteit bij mensen te bestuderen, verder gevalideerd.

**Hoofdstuk 3** is een review van de resultaten van eerdere imaging studies bij ecstasy gebruikers, in het bijzonder studies die gebruik hebben gemaakt van positron emissie tomografie (PET) en SPECT om SERT dichtheden te meten en <sup>1</sup>H-MRS om bepaalde hersenmetabolieten te meten. De meeste PET en SPECT studies leverden suggestief bewijs dat fors ecstasy gebruik geassocieerd is met verlaagde

subcorticale, en mogelijk ook corticale, SERT dichtheden. Deze gevolgen lijken dosis-afhankelijk te zijn en waarschijnlijk (gedeeltelijk) reversibel. Daarnaast zijn vrouwen waarschijnlijk kwetsbaarder voor deze effecten dan mannen. Uit het review bleek dat  $^1\text{H-MRS}$  een minder gevoelige techniek is om de potentiële neurotoxiciteit van ecstasy te bestuderen. De gereviewde studies waren alle retrospectief in opzet en onderzochten hoofdzakelijk forse ecstasy gebruikers. Voor toekomstig op te zetten ecstasy studies adviseren wij om ook de gevolgen van een lage dosering ecstasy gebruik te onderzoeken in een prospectieve setting. Bovendien zijn longitudinale studies nodig om ook meer te kunnen zeggen over de causale rol van ecstasy gebruik in de geobserveerde verschillen in neuroimaging parameters tussen ecstasy gebruikers en niet-gebruikers.

In **hoofdstuk 4** onderzochten wij de validiteit van  $[^{123}\text{I}]\beta\text{-CIT}$  SPECT in het *in vivo* aantonen van MDMA-geïnduceerde neurotoxiciteit bij ratten, waarbij we gebruik maakten van een recent ontwikkelde hoge resolutie pinhole SPECT camera. Wij toonden aan dat na behandeling met MDMA zowel de *in vivo* als ook de *ex vivo*  $[^{123}\text{I}]\beta\text{-CIT}$  uptake ratios afnamen in de thalamus, maar niet in het striatum. Dit suggereert dat  $[^{123}\text{I}]\beta\text{-CIT}$  SPECT in staat is om een MDMA-geïnduceerde afname van SERTs aan te tonen en het daarom een veelbelovende techniek zou kunnen zijn om ook prospectieve studies uit te voeren naar MDMA-geïnduceerde serotonerge neurotoxiciteit in levende kleine dieren.

Hoewel  $[^{123}\text{I}]\beta\text{-CIT}$  SPECT reeds gebruikt wordt om SERT dichtheden in het menselijke brein te bestuderen, bestond er nog discussie over de validiteit van deze methode omdat  $[^{123}\text{I}]\beta\text{-CIT}$  niet alleen bindt aan SERTs, maar ook aan dopamine transporters (DATs). In **hoofdstuk 5** onderzochten we de validiteit van  $[^{123}\text{I}]\beta\text{-CIT}$  SPECT om *in vivo* SERT dichtheden te bepalen in zowel SERT-rijke als ook SERT-arme gebieden in het menselijke brein d.m.v. een dubbelblind, placebo-gecontroleerd, crossover studie design met de selectieve serotonine reuptake inhibitor (SSRI) citalopram. We vonden dat citalopram een afname veroorzaakt van de  $[^{123}\text{I}]\beta\text{-CIT}$  uptake ratios in de SERT-rijke midbrain en (hypo)thalamus. De uptake ratios na citalopram waren ook lager in SERT-arme corticale gebieden, maar dit was alleen statistisch significant in bepaalde corticale gebieden met voxel-by-voxel analyse en niet met een region of interest (ROI) analyse. Daarnaast toonden wij aan dat citalopram een toename veroorzaakte van de uptake ratio's in het DAT-rijke striatum. De resultaten tonen aan dat  $[^{123}\text{I}]\beta\text{-CIT}$  SPECT een valide techniek is om de *in vivo* SERT binding te meten in SERT-rijke gebieden van het menselijke brein. Hoewel enig bewijs werd geleverd dat  $[^{123}\text{I}]\beta\text{-CIT}$  SPECT ook kan worden toegepast om SERTs in SERT-arme corticale gebieden te meten, moeten deze metingen voorzichtig worden geïnterpreteerd.

Het feit dat  $[^{123}\text{I}]\beta\text{-CIT}$  niet selectief aan SERTs bindt, maar ook aan DATs is een nadeel wanneer men het serotonine systeem wil bestuderen, zoals bij ecstasy

gebruikers. Daarom is er de nieuwe radiotracer [ $^{123}\text{I}$ ]ADAM ontwikkeld met een hoge affiniteit voor SERTs, maar niet voor andere transporters zoals DATs. Hiermee is het mogelijk SERTs selectiever te bestuderen. In **hoofdstuk 6** onderzochten wij het tijdsverloop van [ $^{123}\text{I}$ ]ADAM binding aan centrale SERTs bij jong volwassenen met als doel een optimaal tijdstip voor toediening te bepalen. Het tijdstip van piek-specifieke [ $^{123}\text{I}$ ]ADAM binding was erg variabel tussen de vrijwilligers, maar specifieke binding in de SERT-rijke (hypo) thalamus werd bij alle vrijwilligers bereikt binnen 5 uur postinjectie (p.i.). Bovendien was er in dit gebied geen significante verandering in de verhouding van specifieke tot niet-specifieke binding tussen 3 en 6 uur p.i. en bereikte deze verhouding zijn piek 5 uur p.i.. Daarom suggereren wij dat 5 uur p.i. een optimaal tijdstip is voor single-scan [ $^{123}\text{I}$ ]ADAM SPECT studies bij de mens.

### Deel III: Retrospectieve studies bij forse ecstasy gebruikers

Er wordt nog steeds discussie gevoerd of de eerder gemelde neurotoxische effecten worden veroorzaakt door ecstasy, door andere drugs of door een combinatie van verschillende drugs<sup>3,28-32</sup>. Daarom trachtten wij in **hoofdstuk 7** de specifieke/ onafhankelijke effecten van ecstasy en de relatieve bijdragen van amfetamine, cocaïne en cannabis op de hersenen te bestuderen en te onderscheiden in een studiepopulatie met variatie in type en hoeveelheid van gebruikte drugs met een combinatie van  $^1\text{H}$ -MRS, DTI, PWI en [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT. Fors ecstasy gebruik had geen effect op hersenmetabolieten gemeten met  $^1\text{H}$ -MRS en DTI-afgeleide ADC. Fors ecstasy gebruik was echter wel geassocieerd met een lagere FA in de thalamus, een hogere rrCBV in de thalamus en temporale grijze stof en lagere [ $^{123}\text{I}$ ] $\beta$ -CIT binding in de thalamus, de frontale grijze stof en de temporale grijze stof. Na correctie voor het gebruik van andere drugs dan ecstasy en voor potentiële confounders (geslacht, verbale IQ, roken) was er nog een significant specifiek effect van ecstasy op de imaging parameters in de thalamus. Amfetamine en cocaïne gebruik hadden een significant effect op sommige uitkomst parameters in verschillende hersengebieden buiten de thalamus, maar deze bevindingen waren minder consistent en convergerend dan de robuuste bevindingen die gevonden werden bij ecstasy gebruik. Cannabis gebruik had op geen van de uitkomst parameters een significant effect. Deze studie levert daarmee sterk convergerend bewijsmateriaal voor een specifiek toxisch effect van ecstasy op de serotonergic axonen in de thalamus met afname van [ $^{123}\text{I}$ ] $\beta$ -CIT binding, waarschijnlijk veroorzaakt door schade aan de uiteinden van de serotonerge axonen, met een daarmee samenhangende afname van FA door schade aan axonen en een verhoogde rrCBV door vasodilatatie veroorzaakt door een aanhoudende depletie van serotonine.

Omdat serotonine belangrijk is voor vele neurocognitieve en psychopathologische processen, zoals geheugen en stemming<sup>33-36</sup> onderzochten wij ook potentiële klinische gevolgen van ecstasy gebruik. In **hoofdstuk 8** onderzochten wij met behulp van de BDI en het 'composite international diagnostic interview' (CIDI) de effecten van ecstasy gebruik op de stemming, met name in relatie tot SERT densiteiten, gemeten met [<sup>123</sup>I]β-CIT SPECT, ecstasy dosering en geslacht. De prevalentie van klinische depressie gemeten met de CIDI verschilde niet tussen de groepen van gematigde ecstasy gebruikers, forse ecstasy gebruikers, voormalig forse ecstasy gebruikers en drugs-gebruikende maar ecstasy-naïeve controles. Voormalig forse ecstasy gebruikers scoorden echter hoger op BDI dan ecstasy-naïeve controles. Ook was het totale (lifetime) aantal ecstasy tabletten geassocieerd met een hogere BDI score voor depressieve stemming. Wij vonden geen relatie tussen depressieve stemming bij ecstasy gebruikers en geslacht of een afname in SERT dichtheden.

In **hoofdstuk 9** onderzochten we het effect van gematigd, fors en voormalig ecstasy gebruik op cognitieve functies. Aangezien vrouwen kwetsbaarder lijken te zijn voor de gevolgen van ecstasy dan mannen<sup>37-39</sup> en aangezien serotonine transporters belangrijk zijn voor de regulatie van synaptische serotonine transmissie, onderzochten we of de gevolgen van ecstasy gebruik voor cognitie, dat gemeten werd m.b.v. neuropsychologische tests, verschillend is voor vrouwen en mannen en voor personen met een verschillend polymorfisme in het promotorgene van de serotonine transporters (5-HTTLPR). De forse en voormalige ecstasy gebruikers scoorden slechter op geheugentaken dan controles, terwijl de gematigde ecstasy gebruikers even goed scoorden als de controles. Er was tussen de groepen geen verschil in reactietijden of in aandacht / executief functioneren. Wij vonden ook geen significant effect van 5-HTTLPR of geslacht op testprestaties.

## Deel IV: Prospectieve studies in incidentele (low dose) ecstasy gebruikers

In deel IV beschrijven wij de prospectieve substudies van de NeXT studie, waarin voor het eerst prospectief onderzoek werd gedaan naar de aanhoudende gevolgen van ecstasy bij nieuwe gebruikers. Hiervoor werd een groep van 188 ecstasy-naïeve vrijwilligers met een hoog risico voor toekomstig ecstasy gebruik onderzocht bij baseline waarna zij gedurende een periode van ongeveer 18 maanden gevolgd werden. In **hoofdstuk 10** werden de eerste 30 incidente ecstasy gebruikers onderzocht vóór en vrij spoedig na hun eerste ecstasy gebruik (gemiddeld 1.8 ecstasy tabletten) met een combinatie van <sup>1</sup>H-MRS, DTI, PWI en zelfrapportage psychopathologie vragenlijsten. Omdat hersenmetabolieten en FA, beide parameters van structurele neuronale schade, niet veranderden na ecstasy gebruik vonden wij geen aanwijzingen dat incidenteel ecstasy gebruik tot uitgebreide axonale schade leidt. We vonden echter wel een aanhoudende

afname van rrCBV in de thalamus, de dorsolaterale frontale cortex en de superior-occipitale cortex en een afname van ADC in de thalamus na ecstasy gebruik. Dit kan erop wijzen dat zelfs een lage dosis ecstasy aanhoudende vasoconstrictie kan veroorzaken in sommige hersengebieden, hoewel het nog onduidelijk is of dit effect permanent is. Deze bevindingen moeten echter gerepliceerd worden in aanvullende studies, omdat na een correctie voor multiële vergelijkingen slechts de rrCBV daling in de dorsolaterale frontale cortex statistisch significant bleef. Wij vonden na ecstasy gebruik ook een kleine maar significante stijging in impulsiviteit scores en een kleine maar significante daling van depressiescores.

Aan het eind van de follow-up periode onderzochten we de effecten van ecstasy gebruik voor de hersenen met SPECT en MR imaging parameters en zelfrapportage psychopathologie vragenlijsten door 59 incidente ecstasy gebruikers (gemiddeld gebruik van 6.0 tabletten) te vergelijken met 56 persisterend ecstasy-naïeve controles (personen die tijdens de follow-up periode geen ecstasy zijn gaan gebruiken). De vergelijkingen werden gecorrigeerd voor baseline metingen. In **hoofdstuk 11** wordt beschreven dat we in vergelijking met persisterend ecstasy-naïeve vrijwilligers, bij de nieuwe -hoofdzakelijk lage dosering- ecstasy gebruikers een verlaagde FA, verhoogde ADC en verlaagde rrCBV vonden in bepaalde hersengebieden, hoofdzakelijk in de basale ganglia. Dit zou kunnen betekenen dat een lage ecstasy dosis aanhoudende vasoconstrictie en waarschijnlijk ook schade aan axonen van hersenzenuwen veroorzaakt. Hoewel wij geen veranderingen waarnamen in SERT dichtheden en neurometaboliëten suggereren deze resultaten dat ecstasy gebruik zelfs in lage doseringen gevolgen kan hebben voor de hersenen.

In **hoofdstuk 12** onderzochten we het verband tussen ecstasy gebruik en zelfgerapporteerde depressie, impulsiviteit en spanningsbehoefte in (vrijwel) dezelfde prospectieve studiepoulatie. Wij vonden dat depressie, impulsiviteit en spanningsbehoefte geen voorspellers waren voor toekomstig eerste keer ecstasy gebruik in deze poulatie van jong volwassenen met de intentie om ecstasy te gaan gebruiken. Tijdens de follow-up sessie werd een significant effect gevonden van ecstasy gebruik op de algemene en disinhibitie subschalen van de spanningsbehoefte lijst, terwijl er geen effect van ecstasy gebruik werd gevonden op de depressie en impulsiviteit scores. Dit zou betekenen dat gebruik van een lage dosering ecstasy geen depressie of impulsiviteit veroorzaakt, hoewel dit gebruik wel (bepaalde aspecten van) spanningsbehoefte kan verhogen.

## ALGEMENE DISCUSSIE

Het doel van de studies, die in dit proefschrift worden beschreven, was om de kennis van potentiële ecstasy neurotoxiciteit te vergroten, met name op het gebied van nog onbeantwoorde vragen over de causaliteit, het verloop en de klinische relevantie van de potentiële neurotoxiciteit van ecstasy bij mensen. Daarnaast trachtten we de rol van dosis-respons karakteristieken, risico en beschermende factoren en de relatie tussen ecstasy gebruik en het gebruik van andere drugs voor de potentiële neurotoxiciteit van ecstasy te onderzoeken (**hoofdstuk 2**). In aanvulling hierop werd de validiteit van [ $^{123}\text{I}$ ]β-CIT SPECT voor het aantonen van ecstasy-geïnduceerde afname van SERTs geëvalueerd. In deze algemene discussie worden de belangrijkste bevindingen aangehaald in relatie tot de belangrijkste onderzoeksvragen.

### Causaliteit

De causaliteitskwestie, i.e. of er een oorzakelijk verband is tussen ecstasy gebruik en waargenomen verschillen tussen ecstasy gebruikers en niet-gebruikers is zeer complex doordat vrijwel alle onderzoeken gebruik maken van een cross-sectionele en retrospectieve studie opzet, waardoor er gebrek is aan baseline gegevens en er onvoldoende gecontroleerd wordt voor potentiële confounders<sup>8,32</sup>. In dit proefschrift werden drie prospectieve studies beschreven in **hoofdstukken 10, 11, en 12**, welke de eerste prospectieve studies zijn die zich richten op de aanhoudende gevolgen (na een abstinentieperiode van minstens twee weken) van ecstasy gebruik bij nieuwe ecstasy gebruikers door parameters voor en na het eerste ecstasy gebruik met elkaar te vergelijken. Resultaten van beide neuroimaging studies (**hoofdstukken 10 en 11**) lieten geen veranderingen zien in SERT dichtheden ([ $^{123}\text{I}$ ]β-CIT SPECT) en in hersenmetaboliëten ( $^1\text{H}$ -MRS) na het gebruik van lage doseringen ecstasy. Beide studies toonden echter wel een afname van rrCBV waarden, die lijken te wijzen op aanhoudende vasoconstrictie zelfs na een lage dosering ecstasy gebruik, hoewel de hersengebieden met significante rrCBV afname verschilden tussen beide studies. Moeilijker te interpreteren zijn de DTI resultaten, omdat FA in het centrum semiovale tijdens de tussentijdse follow-up sessie verhoogd was (**hoofdstuk 10**) terwijl deze bij de afsluitende follow-up sessie juist verlaagd was (**hoofdstuk 11**). Daarnaast werd er tijdens de afsluitende follow-up sessie een afname van FA in de thalamus gevonden. Hoewel wij veronderstellen dat de verlaagde FA wijst op axonale schade in dit gebied, moeten deze bevindingen voorzichtig worden geïnterpreteerd. Ook vonden we een discrepantie in ADC in de thalamus tussen beide follow-up sessies, waarbij de ADC bij de tussentijdse follow-up sessie verlaagd was terwijl deze bij de afsluitende follow-up juist verhoogd was ten opzichte van de baseline, hoewel deze laatstgenoemde verhoging slechts een relatieve verhoging betrof in vergelijking met persisterende

ecstasy-naïeve vrijwilligers. In dezelfde prospectieve studiegroep vonden we bij de tussentijdse follow-up sessie een kleine maar significante toename in impulsiviteit en een afname in depressie na ecstasy (**hoofdstuk 10**), hoewel dit niet bevestigd werd in de afsluitende follow-up sessie toen vergeleken werd met persisterende ecstasy-naïeven (**hoofdstuk 12**). Aan de andere kant suggereerde deze afsluitende follow-up sessie dat ecstasy gebruik bepaalde aspecten van spanningsbehoefte zou kunnen verhogen.

Het voordeel van de prospectieve opzet is natuurlijk de mogelijkheid om dezelfde parameters voor en na het eerste gebruik van ecstasy te vergelijken bij dezelfde groep vrijwilligers. Bovendien werden de incidentie ecstasy gebruikers en de persisterende ecstasy-naïeve vrijwilligers gerekruteerd middels dezelfde procedure en waren beide groepen bij baseline zeer vergelijkbaar wat betreft potentiële confounders. Daarnaast was het (potentieel confounding) gebruik van amfetamines en cocaïne in de prospectieve populatie zeer laag. Daarom is het zeer waarschijnlijk dat de geobserveerde veranderingen in hersenperfusie en mogelijk ook de veranderingen in diffusie en psychopathologie inderdaad veroorzaakt worden door ecstasy gebruik. Aan de andere kant is het, ondanks de prospectieve opzet, toch nog onmogelijk om met zekerheid vast te stellen of de verbanden daadwerkelijk oorzakelijk zijn, vooral omdat het niet mogelijk is om een perfect gerandomiseerde studie uit te voeren naar ecstasy-geïnduceerde neurotoxiciteit bij mensen. Daarom is het mogelijk dat residuele confounding verantwoordelijk is voor (een deel van) de waargenomen verschillen, wat de causale interpretatie belemmert.

Het nadeel van de prospectieve opzet is dat de meeste vrijwilligers tijdens de follow-up periode slechts eenmalig of enkele keren experimenteerden met het gebruik van ecstasy, zodat de gemiddelde cumulatieve doses klein waren met gemiddelden van slechts 1.8 (**hoofdstuk 10**) en 6.0 tabletten (**hoofdstukken 11 en 12**) waarbij slechts enkele vrijwilligers meer dan 10 ecstasy tabletten hebben gebruikt. Daarom onderzochten wij de gevolgen van fors ecstasy gebruik met behulp van een retrospectieve studie opzet waarbij we gebruik maakten van dezelfde imaging parameters. Hoewel het met een retrospectieve studie niet mogelijk is om causaliteit vast te stellen werd in **hoofdstuk 7** een sterke en convergerende relatie gevonden tussen ecstasy gebruik en veranderingen in imaging parameters in de thalamus. Met behulp van lineaire regressieanalyses in een groep vrijwilligers met variaties in soort en hoeveelheid gebruikte drugs was het mogelijk om correlaties tussen verschillende drugs te minimaliseren en om tot op zekere hoogte onderscheid te maken tussen de effecten van ecstasy en van andere drugs. Ook in de retrospectieve studie van **hoofdstuk 8** werd een causale relatie gesuggereerd tussen ecstasy gebruik en verhoogde depressieve symptomen door de significante correlatie tussen de cumulatieve ecstasy dosis en hogere BDI scores.

## Beloop

Een andere onderzoeksvraag betrof het lange termijn beloop van ecstasy geïnduceerde neurotoxiciteit en gerelateerde klinische consequenties. **Hoofdstuk 8** toonde hogere depressie scores, gemeten met de BDI, bij voormalig forse ecstasy gebruikers die tenminste 1 jaar abtinent waren, hoewel dit niet geassocieerd was met dalingen in SERT dichtheden. Een eerdere studie bij zelfde studiepopulatie toonde significant lagere SERT dichtheden bij vrouwelijke huidige forse ecstasy gebruikers, maar niet bij mannen of bij voormalig forse ecstasy gebruikers<sup>39</sup>, waardoor het waarschijnlijk lijkt dat SERTs zich herstellen, een bevinding die onlangs door anderen werd bevestigd<sup>37</sup>. Echter, zoals aangetoond in **hoofdstuk 9** en in een vorige publicatie in dit cohort<sup>40</sup>, blijken zowel mannelijke als vrouwelijke voormalig forse ecstasy gebruikers slechter te presteren op een verbale geheugentaak dan niet-gebruikers. Het zou mogelijk kunnen zijn dat, ondanks dat SERTs zich lijken te herstellen, deze transporters niet optimaal functioneren, bijvoorbeeld door abnormale sprouting van de resterende serotonerge neuronen of door up-regulatie van SERTs in de resterende serotonerge axonen<sup>41</sup>.

Omdat de follow-up periode in het prospectieve cohort beperkt was tot ongeveer 18 maanden kunnen wij geen conclusies trekken wat betreft de lange termijn effecten van ecstasy gebruik in dit cohort. Voor het beantwoorden van deze vraag zouden we het prospectieve cohort ook in de toekomst moeten vervolgen. De retrospectieve cohort substudie van de NeXT studie, bij lifetime ecstasy gebruikers en gematchte controles van een bestaand epidemiologisch cohort (**hoofdstuk 2**) zal meer informatie geven over het beloop en de gevolgen van ecstasy gebruik in de algemene populatie op de langere termijn, maar resultaten van deze substudie worden niet beschreven in dit proefschrift.

## Klinische relevantie

De klinische consequenties van de verlaagde rrCBV waarden en mogelijk ook veranderde diffusie na lage dosering ecstasy gebruik zijn niet nog duidelijk, vooral omdat we niet weten of de gevolgen aanhoudend maar reversibele zijn of dat ze permanent zijn. Bovendien, zoals hierboven besproken, lijken de gevolgen van een lage dosering ecstasy voor de psychopathologie vrij subtiel te zijn (**hoofdstukken 10 en 12**). Ook liet functioneel MRI (fMRI) onderzoek in hetzelfde cohort geen duidelijk bewijs zien voor aanhoudende effecten van een lage dosis ecstasy op het werkgeheugen, de selectieve aandacht of op het associatieve geheugen, noch op het gedragsniveau maar ook niet op het neurofysiologische niveau (niet in dit proefschrift)<sup>42</sup>. Aan de andere kant toonde een neuropsychologische studie in hetzelfde prospectieve cohort aan dat zowel de directe als ook de delayed verbale recall en de verbale herkenning bij

incidente ecstasy gebruikers daalden, relatief ten opzichte van niet gebruikers (niet in dit proefschrift) <sup>43</sup>.

Het is mogelijk dat klinische consequenties pas evident worden na fors ecstasy gebruik, zoals ook bevestigd wordt in **hoofdstukken 8 en 9**, waarin toegenomen depressieve symptomen, gemeten met de BDI, worden beschreven bij voormalig forse ecstasy gebruikers en slechtere verbale geheugenprestaties bij forse en voormalig forse ecstasy gebruikers in vergelijking tot niet-gebruikers. Aan de andere kant werd er geen effect van ecstasy waargenomen op klinische tekenen van depressie zoals die gemeten werd met de CIDI en ook niet op metingen van reactietijden, aandacht of op het executief functioneren. De klinische relevantie van de specifieke effecten van ecstasy op de imaging parameters in de thalamus die werden beschreven in **hoofdstuk 7** is ook nog niet duidelijk. De fMRI bevindingen in dezelfde populatie laten echter zien dat ecstasy gebruik geassocieerd was met verminderde performance en veranderde hersenactiviteit op het gebied van associatief geheugen, hoewel het effect van amfetamine op de associatieve geheugenprestaties veel sterker was dan het effect van ecstasy. Ecstasy en ander druggebruik hadden weinig effect op het werkgeheugen en aandachtsniveau (niet in dit proefschrift) <sup>44</sup>. Het neuropsychologische onderzoek in dezelfde groep toonde een specifiek aanhoudend negatief effect van ecstasy gebruik op het verbale geheugen, hoewel de klinische relevantie hiervan niet onmiddellijk duidelijk is, omdat de testprestaties over het algemeen binnen de norm bleven (niet in dit proefschrift) <sup>45</sup>. Aangezien alle ecstasy gebruikers in deze studiepopulatie ecstasy gebruikten hadden in de 6 maanden voorafgaande aan het onderzoek, is het nog niet duidelijk of deze gevolgen permanent zijn.

## Dosis-respons karakteristieken

De range van de cumulatieve doses ecstasy tabletten is waarschijnlijk te klein, zowel in de prospectieve studies van **hoofdstukken 10 tot 12** (bijna uitsluitend low dose gebruikers) als ook in studie bij forse ecstasy gebruikers van **hoofdstuk 7** (bijna uitsluitend forse ecstasy gebruikers), om dosis-respons effecten in de afzonderlijke studies aan te tonen. Er werden echter verschillen gevonden in de resultaten van beide studiepopulaties die mogelijk verklaard zouden kunnen worden door het verschil in cumulatieve ecstasy doses tussen beide studies, vooral omdat in beide cohorten precies dezelfde imaging technieken werden toegepast. Terwijl er geen verandering in SERT dichtheid, gemeten met [<sup>123</sup>I]β-CIT SPECT, werd waargenomen in de lage dosis gebruikers (**hoofdstuk 11**), vonden we wel een verlaagde SERT dichtheid in de thalamus bij forse ecstasy gebruikers (**hoofdstuk 7**), zodat de verminderde SERT dichtheid dosis afhankelijk lijkt te zijn. Dit werd ook aangetoond in eerdere studies <sup>39,46</sup>. Een daling van FA in de thalamus werd zowel gevonden bij lage dosering ecstasy gebruikers (**hoofdstuk 11**) evenals bij forse ecstasy gebruikers (**hoofdstuk 7**). De dosis-respons relatie tussen

ecstasy gebruik en rrCBV waarden is moeilijker te interpreteren, omdat de low dose studies hoofdzakelijk een verminderde perfusie lieten zien (**hoofdstukken 10 en 11**), terwijl de studie bij forse ecstasy gebruikers in sommige hersengebieden juist een toegenomen perfusie liet zien (**hoofdstuk 7**). Eerder werd al gesuggereerd dat er mogelijk een verband zou kunnen bestaan tussen rrCBV waarden en de tijd sinds het laatste ecstasy gebruik<sup>47-49</sup>, hoewel wij dit in onze studies niet konden bevestigen. Deze schijnbare inconsistentie zou mogelijk kunnen betekenen dat er een complex, nog op te helderen, verband is tussen ecstasy gebruik en de door serotonine geregelde hersenperfusie. Dit verband zou mogelijk gerelateerd zijn aan de tijd sinds laatste ecstasy inname, de cumulatieve dosis en aan adaptatie van serotonerge transporters en receptoren aan de ecstasy-geïnduceerde serotonine toename in het (sub)acute stadium en de serotonine depletie op de lange termijn.

We vonden een dosis-respons relatie tussen cumulatieve ecstasy dosis en verhoogde depressieve symptomen gemeten met BDI (**hoofdstuk 8**). Hoewel de tussentijdse follow-up sessie van de prospectieve studie ook een kleine maar significant verhoogde BDI score liet zien na lage dosering ecstasy gebruik (**hoofdstuk 10**), werd dit niet bevestigd in de afsluitende follow-up sessie (**hoofdstuk 12**). In **hoofdstuk 9** lijkt er sprake te zijn van een dosis-respons relatie tussen het verbale geheugen en ecstasy dosis, aangezien forse en voormalig forse ecstasy gebruikers slechter presteerden op een verbale geheugentaak, terwijl de gematigde ecstasy gebruikers dit niet deden.

## Risico en beschermende factoren

Voorgaande studies wezen erop dat vrouwen kwetsbaarder lijken te zijn voor de gevolgen van ecstasy dan mannen<sup>37-39</sup> (zie ook het review artikel in **hoofdstuk 3**). In de huidige studies namen wij echter op geen enkele uitkomstparameter een duidelijk effect waar van geslacht. Wij vonden geen aanwijzing dat de korte allel variant van het serotonine transporter promotor gen (5-HTTLPR) een risico factor zou zijn voor de effecten van ecstasy op cognitie (**hoofdstuk 9**), hoewel eerder wel een kwetsbaarheids effect van de korte variant van de 5-HTTLPR werd gevonden op abnormale emotionele verwerking bij ecstasy gebruikers<sup>50</sup>. Wij konden ook niet bevestigen dat verhoogde symptomen van depressie, impulsiviteit of spanningsbehoefte voorspellers zouden kunnen zijn voor toekomstig eerste keer ecstasy gebruik (**hoofdstuk 12**).

## De rol van ander druggebruik bij de potentiële neurotoxiciteit van ecstasy gebruik

Één van de belangrijkste problemen van ecstasy onderzoek is dat de pure ecstasy gebruiker nauwelijks bestaat<sup>51-53</sup>. Daarom is het belangrijk dat de potentiële confounding effecten van andere drugs dan ecstasy in acht worden genomen,

alhoewel ecstasy de enige drug is met een selectief effect op het serotonine systeem. De specifieke onderzoeksvraag naar het verband tussen ecstasy gebruik en gebruik van andere drugs bij de potentiële neurotoxiciteit van ecstasy werd onderzocht in **hoofdstuk 7**. Wij vonden een significant en specifiek effect van fors ecstasy gebruik op diverse neuroimaging parameters in de thalamus, onafhankelijk van andere drugs. In dezelfde studie vonden we dat amfetamine en cocaïne significante effecten hadden op sommige uitkomstparameters in hersengebieden buiten de thalamus, maar deze bevindingen waren minder consistent en convergerend dan de bevindingen gerelateerd aan ecstasy gebruik. Cannabis had op geen enkele uitkomstparameter een significant effect.

Een voordeel van de prospectieve studies (**hoofdstukken 10 tot 12**) was dat de vrijwilligers behalve ecstasy nauwelijks andere harddrugs gebruikten en dat er tot nu toe geen hard bewijs bestaat voor blijvende gevolgen van cannabisgebruik voor de hersenen, vooral niet in de gematigde doseringen zoals die door onze vrijwilligers gebruikt werden.

## Methodologie, meettechnieken en confounders

In **hoofdstuk 2** werd besproken dat de belangrijkste onderzoeksvragen over causaliteit, beloop en klinische relevantie van de potentiële neurotoxiciteit van ecstasy met name ontstaan zijn vanuit de methodologische beperkingen inherent aan naturalistische humane studies op dit onderzoeksgebied. Dit betreft met name inadequate ‘sampling’ van gebruikers en controles, kleine groepen, gebrek aan goede analyse van druggebruik, beperkte spreiding in het aantal pillen, korte follow-up periodes en cross-sectionele and retrospectieve studie designs zonder baseline gegevens en met ontoereikende controle voor potentiële confounders<sup>8,32,54-56</sup>. De sterke en zwakke punten van de studies die in dit proefschrift worden beschreven werden reeds uitgebreid in elk afzonderlijk hoofdstuk besproken en daarom geven we hier slechts een korte samenvatting en discussie.

Het moge duidelijk zijn dat de opzet van de studies één van de sterkste punten van dit proefschrift is. Ten eerste werd het gebruik van [<sup>123</sup>I]β-CIT SPECT om SERT dichtheden te meten uitgebreid gevalideerd en gereviewed (**hoofdstukken 3 tot 5**). In de cross-sectionele substudie bij forse ecstasy gebruikers pasten wij een nieuwe benadering toe om te corrigeren voor confounding door polydrug gebruik (**hoofdstuk 7**). De meest innovatieve opzet werd toegepast bij de prospectieve substudies van **hoofdstukken 10 tot 12**. Voor zover ons bekend is de NeXT studie de eerste en op dit moment nog enige die erin geslaagd is om bij zo’n grote groep nieuwe ecstasy gebruikers metingen te verrichten en te vergelijken voor en na hun eerste ecstasy gebruik. Een ander pluspunt van de huidige studie is dat wij verschillende imaging technieken, psychopathologie vragenlijsten en neuropsychologisch

onderzoek gecombineerd hebben om zo verschillende aspecten van potentiële neurotoxiciteit te kunnen beoordelen. Naast de onderzoeken die in dit proefschrift werden besproken, werd ook neuropsychologisch en fMRI onderzoek uitgevoerd bij dezelfde onderzoeksgroepen van de substudie bij forse ecstasy gebruikers en van de prospectieve substudie van de NeXT studie <sup>42-45</sup>.

De beperkingen van de studies die in dit proefschrift aan de orde kwamen hangen samen met het gebrek aan controle op de dosis en zuiverheid van de ingenomen ecstasy tabletten, de selectie van vrijwilligers, de potentiële confounding effecten van polydrug gebruik, de imaging technieken en correctie voor multipale vergelijkingen.

Inherent aan de naturalistische benadering van onze studies, bestaat er onzekerheid over mogelijke variaties in dosering en zuiverheid van ingenomen ecstasy tabletten. Tablet analyses bevestigen echter dat in Nederland meer dan 95% van de tabletten die als ecstasy worden verkocht MDMA bevatten als enige (91.2%) of belangrijkste (4.2%) component <sup>57-60</sup>. De gemiddelde concentratie van MDMA in een ecstasy tablet bedroeg in 2003 in Nederland 78 mg <sup>59</sup>. Ook zullen de omgevingsomstandigheden waarin ecstasy werd gebruikt en het gelijktijdige gebruik van andere drugs heterogeen zijn geweest. De frequentie en de hoeveelheid van bepaald druggebruik werd hoofdzakelijk vastgesteld aan de hand van zelfrapportage vragenlijsten. De abstinentie periode van bepaald druggebruik vóór de onderzoeken werd geverifieerd met behulp van urineanalyses, hoewel de gevoeligheid van deze testen beperkt is, aangezien voor de meeste drugs de urinetest alleen positief zal zijn wanneer de drugs in de laatste dagen werden genomen.

Ook inherent aan dit onderzoekveld, werden vrijwilligers geselecteerd en niet willekeurig uitgekozen, iets wat de generalisatie van onze bevindingen zou kunnen beperken. De vrijwilligers namen deel aan een nogal uitgebreid onderzoekproject met o.a. hersenscans, neuropsychologisch onderzoek en afname van bloedmonsters. Dit heeft waarschijnlijk gezorgd voor selectie van sterk gemotiveerde vrijwilligers. Het viel ons ook op dat hoger opgeleide vrijwilligers eerder bereid waren deel te nemen aan onze studies dan lager opgeleide personen. Vanwege onze selectiestrategie bij de NeXT substudie naar forse ecstasy gebruikers (**hoofdstuk 7**) hebben we selectieve ecstasy gebruikers en polydrug controles zonder ervaring met ecstasy gebruik geïncludeerd, die atypisch zijn en mogelijk niet-representatief voor een grotere groep. Daarnaast werden de vrijwilligers niet willekeurig aan de verschillende studies toegewezen, zodat behalve ecstasy gebruik ook residuele confounding een deel van de verschillen tussen ecstasy gebruikers en niet-gebruikers zou kunnen verklaren. Hoewel wij gepoogd hebben om de potentiële confounding effecten te beperken door middel van unieke studie designs en adequate statistische modellen, kunnen we confounding effecten van het gebruik van andere drugs, zoals alcohol, nicotine, cannabis, amfetaminen en cocaïne, niet geheel uitsluiten.

Omdat slechts enkele ecstasy studies gebruik hebben gemaakt van  $^1\text{H-MRS}$ , DTI en PWI om neuronale schade te onderzoeken, is er weinig bekend over de sensitiviteit en de specificiteit van deze technieken om ecstasy-geïnduceerde neuronale schade op te sporen, vooral niet voor het aantonen van relatief kleine veranderingen na gebruik van een lage ecstasy doseringen. Ook is er weinig bekend over de reproduceerbaarheid van de gebruikte MRI en SPECT metingen, vooral niet na een relatief lange follow-up periode van 18 maanden. Hoewel eerdere studies aantoonde dat  $^1\text{H-MRS}$ , DTI en PWI gevoelige technieken kunnen zijn bij diverse neuropsychiatrische stoornissen <sup>4,61-69</sup> en dat de reproduceerbaarheid goed is <sup>70-73</sup>, zijn er aanvullende (dier) studies nodig.

In het grootste deel van onze studies hebben we geen statistische correcties toegepast voor multiële vergelijkingen, hoewel we wel meerdere technieken hebben gebruikt als indicatoren voor ecstasy-geïnduceerde hersenschade en ook meerdere hersengebieden hebben onderzocht. Deze aanpak verhoogt de waarschijnlijkheid van type I fouten (vals positieve resultaten). Wanneer we Bonferroni correcties zouden toepassen, zouden veel van de significante bevindingen niet meer significant zijn, zoals ook in **hoofdstuk 10** werd aangetoond. Aan de andere kant is de Bonferroni correctie waarschijnlijk te conservatief, vooral in de prospectieve studies, omdat we hier *a priori* relatief kleine effecten verwachtten aangezien we vroege indicatoren van potentiële hersenschade onderzochten bij vrijwilligers met slechts *lage* cumulatieve ecstasy doses. Bovendien werden alle imaging technieken en ROI's gekozen op basis van *a priori* hypothesen. Het is daarom waarschijnlijk dat Bonferroni correctie leidt tot type II fouten (vals negatieve bevindingen). Het risico van dergelijke correcties werd eerder besproken door Rothman, die aantoonde dat correctie voor multiële vergelijkingen mogelijk belangrijke bevindingen kan verhullen <sup>74</sup>. Vanwege de grote maatschappelijke impact is aanvullend onderzoek nodig om vast te stellen of onze huidige ongecorrigeerde significante bevindingen gereproduceerd kunnen worden.

## Implicaties

De belangrijkste doelstelling van de NeXT studie was om tot wetenschappelijke conclusies te komen betreffende de neurotoxiciteit van ecstasy, die vervolgens gebruikt kunnen worden voor preventie adviezen, klinische besluitvorming, en de ontwikkeling van een (inter) nationaal ecstasy beleid. Hoewel dit proefschrift slechts een deel beschrijft van de NeXT studie en de bevindingen samen met de andere substudies geïnterpreteerd moeten worden, vóórdat definitieve conclusies kunnen worden getrokken en aanbevelingen kunnen worden opgesteld, willen wij toch in het kort de potentiële implicaties van de bevindingen van dit proefschrift bespreken.

De bevindingen van de studies bij forse ecstasy tonen sterk convergerend bewijs voor een specifiek toxisch effect van ecstasy op serotonerge axonen in de thalamus

(**hoofdstuk 7**), verhoogde symptomen van depressie (**hoofdstuk 8**) en slechtere prestaties van het verbale geheugen (**hoofdstuk 9**). Deze bevindingen bevestigen de algemene bezorgdheid over de negatieve gevolgen van fors ecstasy gebruik op de hersenen en de bijbehorende functionele beperkingen. Daarom zijn we van mening dat volksgezondheidsmaatregelen getroffen zouden moeten worden om fors recreatief ecstasy gebruik zoveel mogelijk te beperken. Voor incidenteel ecstasy gebruik is dit minder duidelijk, omdat wij aanwijzingen vonden voor aanhoudende verminderde perfusie van sommige hersengebieden, maar geen duidelijk bewijs vonden voor structurele hersenschade of relevante klinische gevolgen (**hoofdstukken 10, 11, en 12**). Ook andere studies lieten eerder zien dat de ongunstige effecten van een lage ecstasy dosis beperkt zijn <sup>75,76</sup>. Aan de andere kant lijkt de afname in rCBV te wijzen op verlengde vasoconstrictie en de afname in FA op axonale schade, zelfs na lage dosis ecstasy gebruik. Bovendien suggereren de neuropsychologische resultaten bij dezelfde studiepopulatie, dat zelfs een lage dosis ecstasy gebruik leidt tot een kleine maar significant daling in verbaal geheugen, wanneer vergeleken wordt met niet-gebruikers <sup>43</sup>. Hoewel we niet weten of deze gevolgen permanent zijn, kunnen wij niet concluderen dat incidenteel ecstasy gebruik veilig is voor de menselijke hersenen. Daarnaast zijn er nog diverse factoren (zoals een slechter functionerend metabolisme, hypertensie, jongere leeftijd, het gelijktijdige gebruik van andere middelen en omgeving condities), die mogelijk bijdragen aan individuele en situationele gevoeligheid voor acute nadelige effecten en lange termijn neurotoxiciteit van ecstasy <sup>77-80</sup>. Daarom zijn wij van mening dat huidige gebruikers en potentiële toekomstige gebruikers goed geïnformeerd moeten worden over de potentiële risico's van ecstasy gebruik, zelfs incidenteel ecstasy gebruik. Wat betreft het voorschrijven van MDMA als medicatie bij psychotherapie zouden de potentiële voordelen en risico's zorgvuldig moeten worden afgewogen en meer onderzoek naar dit onderwerp is noodzakelijk.

## Aanbevelingen voor toekomstig onderzoek

De eerstvolgende belangrijke stap is om alle substudies van het NeXT project af te ronden, inclusief de studies die niet in dit proefschrift wordt besproken, zoals de retrospectieve cohort studie en ook de neuropsychologische en fMRI onderzoeken bij de forse ecstasy gebruikers en de prospectieve substudies. De bevindingen van deze studies moeten worden geïntegreerd om tot definitieve conclusies en aanbevelingen van dit ambitieuze project te komen.

Ten tweede zijn wij de eerste die aanhoudende gevolgen van ecstasy rapporteren bij nieuwe incidentele ecstasy gebruikers door middel van een prospectieve studieopzet, zodat het belangrijk is dat onze bevindingen worden gerepliceerd, bij voorkeur ook in grote prospectieve studies. Hiermee samenhangend zijn meer studies nodig naar de acute en lange termijn effecten van incidenteel en gemiddeld ecstasy gebruik, in

plaats van het herhalen van studies bij forse ecstasy gebruikers, vooral ook omdat de meeste jongeren slechts zullen experimenteren met ecstasy, terwijl slechts 20-30% van hen een regelmatige gebruiker zal worden <sup>57</sup>.

Ten derde weten wij van de meeste effecten, gepresenteerd in dit proefschrift, niet of ze permanent zijn, omdat de vrijwilligers recente ecstasy gebruikers waren (in de afgelopen zes maanden gebruikt). Ook relatief weinig andere studies onderzochten de lange termijn gevolgen van ecstasy. Daarom is het belangrijk om kennis te vergroten van de lange termijn effecten van ecstasy. In dit verband zal het zeer interessant zijn om ons huidige prospectieve cohort te blijven vervolgen. Dit cohort creëert een unieke mogelijkheid om de causaliteitskwestie opnieuw te onderzoeken, wegens de te verwachten toename in variatie in dosis, frequentie en duur van ecstasy gebruik binnen deze groep van nieuwe gebruikers. Men zou ook kunnen veronderstellen dat ecstasy gebruik het verouderen van de hersenen versteekt, met name de fysiologische leeftijdsafhankelijke daling van het aantal serotonine cellen. Dit kan betekenen dat de gevolgen van ecstasy, voor bijvoorbeeld het geheugen, pas klinisch relevant worden op latere leeftijd, wanneer de reservecapaciteit van de hersenen is afgenomen. Daarom zouden toekomstige ecstasy studies ook de effecten bij oudere (voormalige) ecstasy gebruikers moeten onderzoeken.

Ten vierde zouden toekomstige studies ook verschillende neuroimaging technieken en neuropsychologische en psychiatrische onderzoeken moeten blijven combineren zodat het mogelijk is convergerend bewijs te verkrijgen. Daarnaast zouden ze zo moeten worden opgezet dat potentiële confounding effecten van levensstijl, demografische gegevens, polydrug gebruik en van preëxistente psychiatrische stoornissen en cognitief disfunctioneren zoveel mogelijk beperkt blijven. Voorts moet als vanzelfsprekend druggebruik in de voorgeschiedenis gedetailleerd worden uitgevraagd en idealiter zou dit bevestigd moeten worden door middel van urine en haaranalyse. Wanneer men aanhoudende (niet-acute) effecten van ecstasy wil bestuderen, moet men een minimum abstinentieperiode van minstens 10 dagen in acht nemen om zo acute farmacologische effecten te vermijden.

Ten vijfde, aangezien het gebied van de neuroimaging zich zeer snel ontwikkelt, zijn er heel wat technische verbeteringen doorgevoerd sinds het begin van het NeXT project in 2001. Terwijl wij in onze studies een MRI scanner gebruikten met een magnetische veld van 1.5T, is in 2007 voor hersenonderzoek 3T 'state of the art' en de verwachting is dat de komende jaren een toenemend aantal 7T scanners zal worden geïmplementeerd voor humaan hersenonderzoek. Theoretisch zal de gevoeligheid van de verschillende MRI technieken om ook meer subtiele tekenen van neurotoxiciteit aan te tonen toenemen met hogere veld MRI scanners, maar dit moet bevestigd worden in toekomstige studies. Terwijl onze huidige resultaten en resultaten van anderen lijken te wijzen op een beperkte sensitiviteit van <sup>1</sup>H-MRS voor het aantonen van veranderingen in neurometabolieten, in ieder geval bij incidenteel

en gemiddeld ecstasy gebruik, zijn de resultaten van hoog veld  $^1\text{H}$ -MRS op andere onderzoeksterreinen veelbelovend. Ook moet de waarde van geavanceerdere technieken, zoals DTI fiber tracking, voor ecstasy onderzoek worden geëvalueerd. Voor het meten van hersenperfusie zal arterial spin labelling mogelijk een belangrijke techniek worden, omdat het met deze techniek mogelijk is om hersendoorbloeding in kwantitatieve in plaats van relatieve maten te meten zonder dat er een contrastmiddel hoeft te worden toegediend. Verder is het zeer waarschijnlijk dat het in de toekomst mogelijk zal zijn om serotonerge functies op een directere manier te onderzoeken met behulp van farmacologische of moleculaire MRI technieken <sup>81</sup>. Wat betreft SERT SPECT imaging zijn selectievere SERT liganden ontwikkeld, zoals het [ $^{123}\text{I}$ ]ADAM (hoofdstuk 6) <sup>82</sup>. Toekomstige studies zullen moeten uitwijzen of deze selectieve liganden sensitiever zijn in het aantonen van ecstasy-geïnduceerde afname in SERT dichtheden, vooral ook in de SERT-arme corticale gebieden. Hoewel deze verbeterde technieken de sensitiviteit zouden kunnen verbeteren, moet men zich realiseren dat inter- en intra- individuele verschillen zullen blijven bestaan.

Tot slot zouden klinische trials met MDMA bij patiënten van belang kunnen zijn (ondanks de heftige debatten of zulke studies ethisch gerechtvaardigd en/of veilig zijn). Op deze manier kunnen we onze kennis vergroten van de effecten van gecontroleerd en lage dosis ecstasy gebruik voor de menselijke hersenen en of de potentiële voordelen van MDMA als hulpmiddel bij psychotherapie zwaarder wegen dan de potentiële risico's.

## CONCLUSIES

Samenvattend beschrijven wij in dit proefschrift retrospectieve en prospectieve studies die potentiële neurotoxische effecten onderzochten van de populaire recreatieve drug ecstasy met behulp van neuroimaging, psychopathologie vragenlijsten en neuropsychologische tests in verschillende groepen ecstasy gebruikers. De onderzoeksvragen waren vooral gericht op het vergroten van kennis over causaliteit, beloop, en klinische relevantie van mogelijk ecstasy gerelateerde (serotonerge) neurotoxiciteit bij mensen. Daarnaast onderzochten wij de validiteit van [ $^{123}\text{I}$ ] $\beta$ -CIT SPECT voor het bestuderen van de SERT. De resultaten toonden aan dat [ $^{123}\text{I}$ ] $\beta$ -CIT inderdaad een valide techniek is om de dichtheid van de SERT te bepalen, met name in SERT-rijke hersengebieden en in mindere mate ook in SERT-arme corticale gebieden. Wij vonden ook dat fors ecstasy gebruik een specifiek effect heeft op de thalamus, onafhankelijk van het gebruik van andere drugs en dat fors ecstasy gebruik waarschijnlijk geassocieerd is met toename van depressieve symptomen en afname van het verbale geheugen. Tot slot lijken de resultaten te wijzen op een oorzakelijk verband tussen lage dosering ecstasy gebruik en aanhoudende veranderingen in perfusie van de hersenen, hoewel we geen sterk bewijs

vonden voor tot axonale schade veroorzaakt door low dose ecstasy gebruik. Klinisch lijkt een lage dosis ecstasy geen toename in depressiviteit of impulsiviteit te veroorzaken, maar lage dosis ecstasy zou mogelijk (sommige) aspecten van spanningsbehoefte kunnen verhogen. De bevindingen in de lage dosering ecstasy gebruikers zouden bevestigd moeten worden in toekomstig onderzoek.

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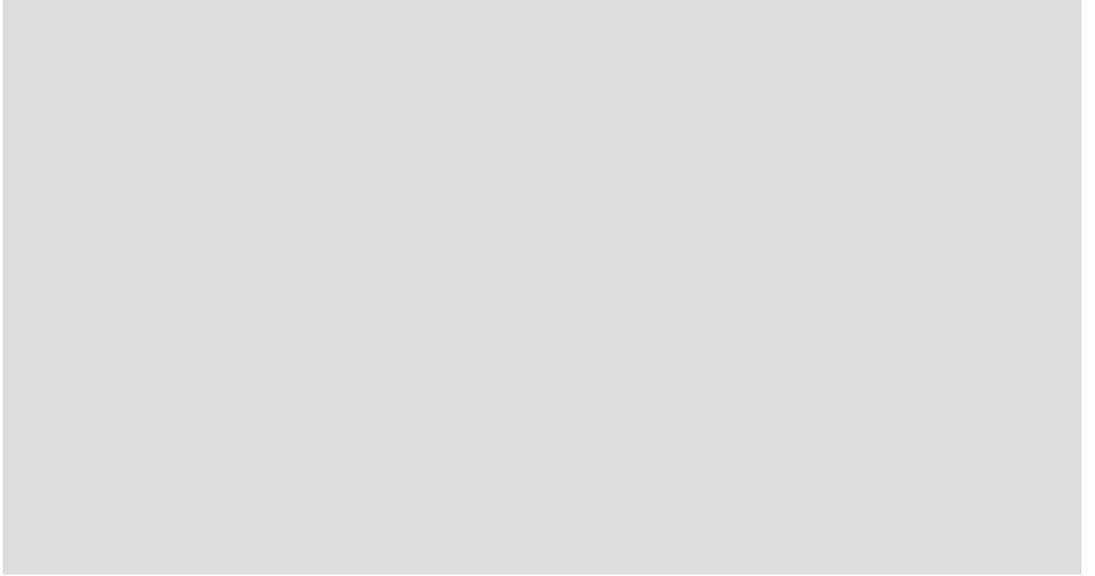
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# Colour Illustrations



## Chapter 4

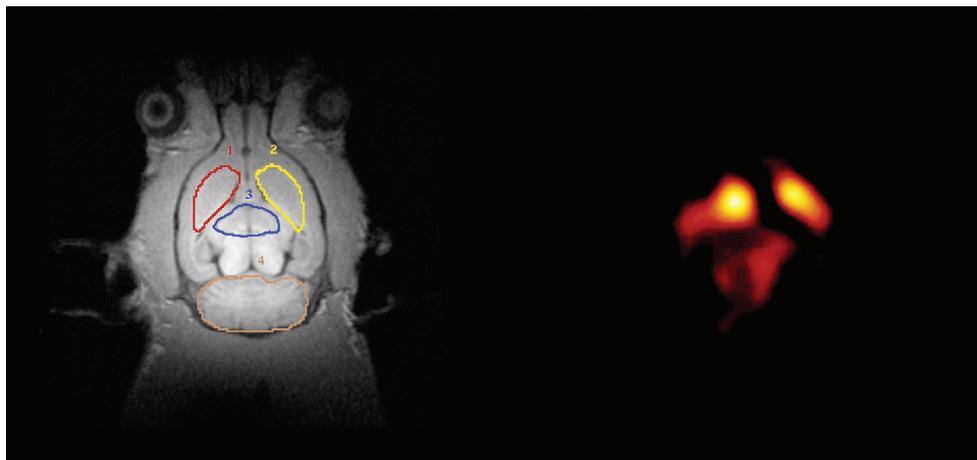


Figure 1

## Chapter 5

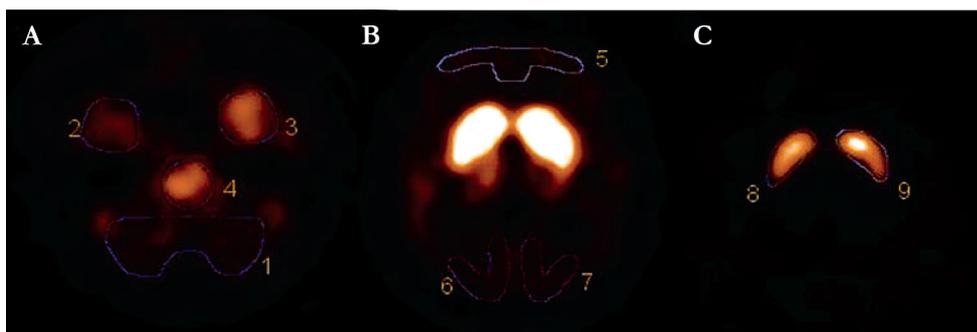


Figure 1



Figure 2

## Chapter 5

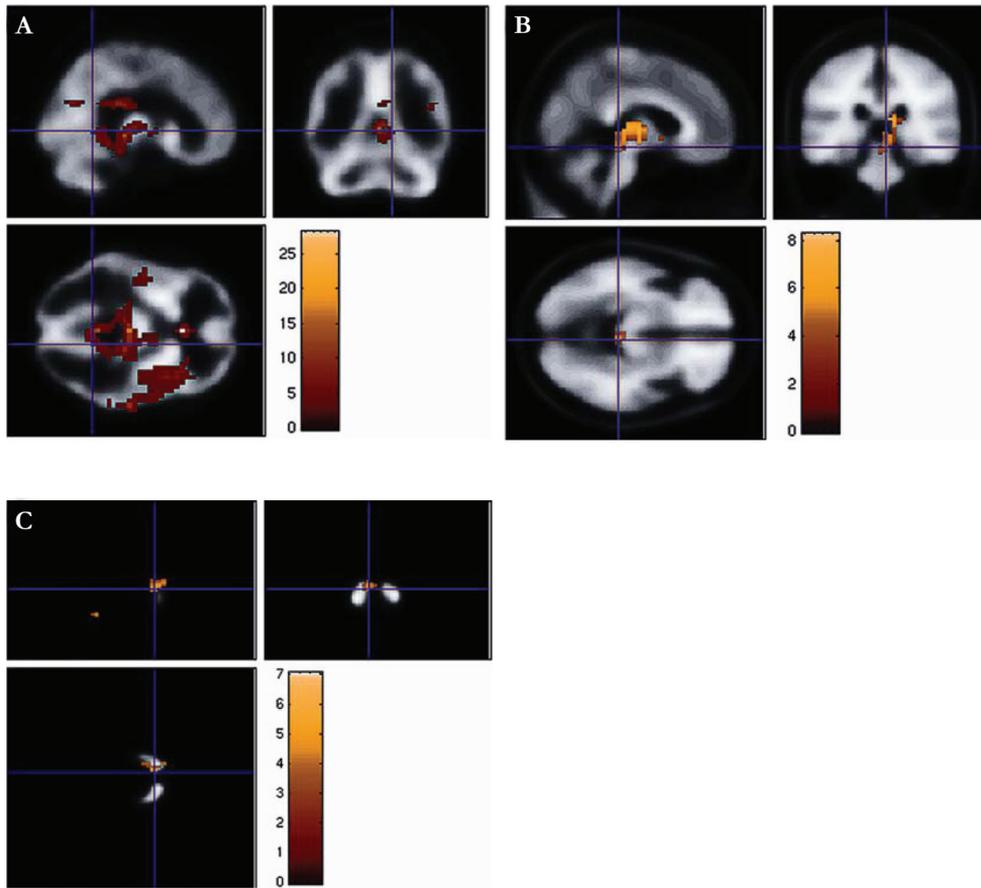


Figure 3

## Chapter 6

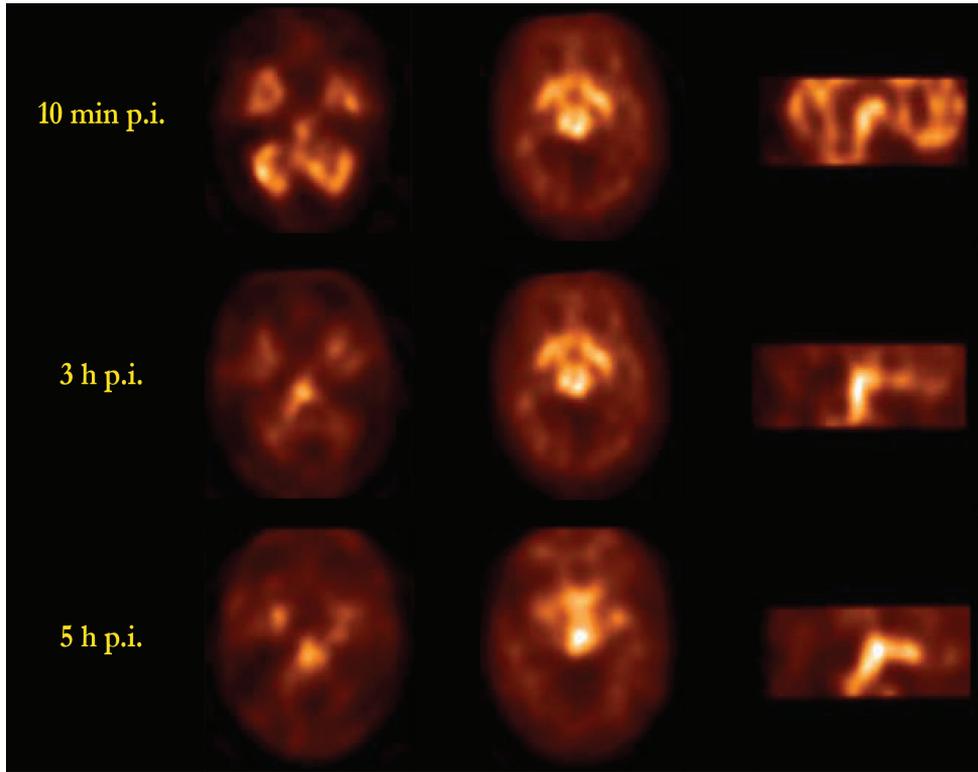


Figure 1

## Chapter 7

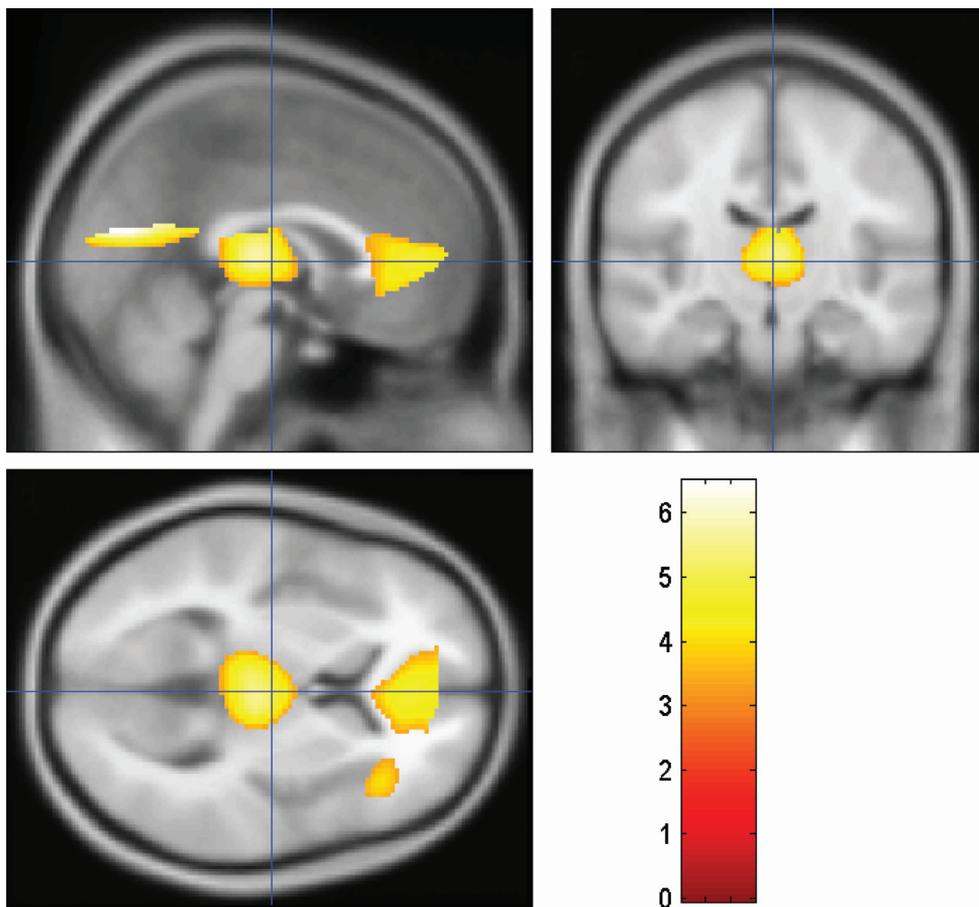
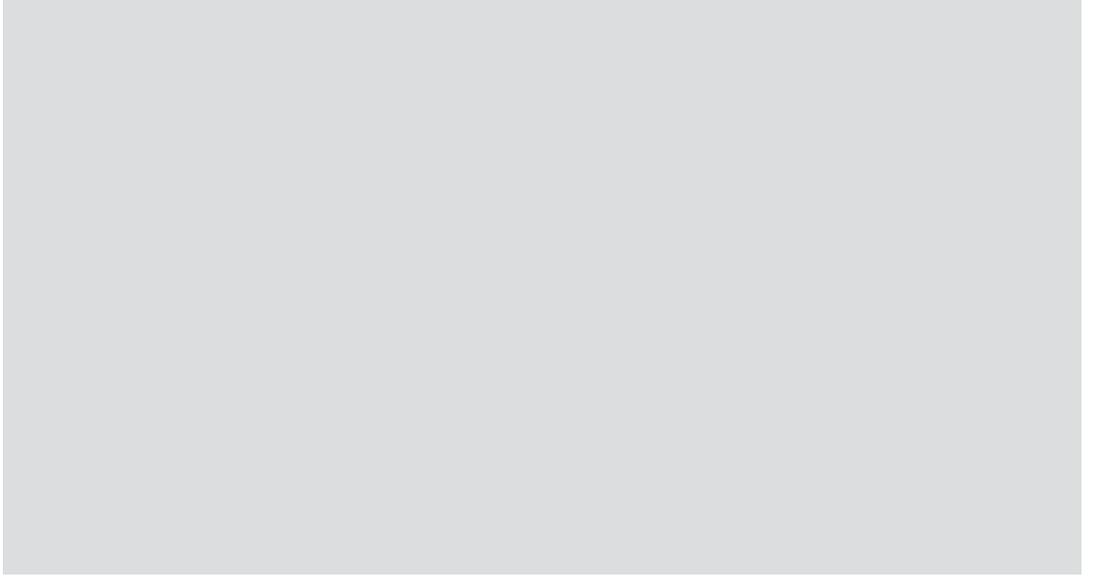


Figure 3





## List of Abbreviations

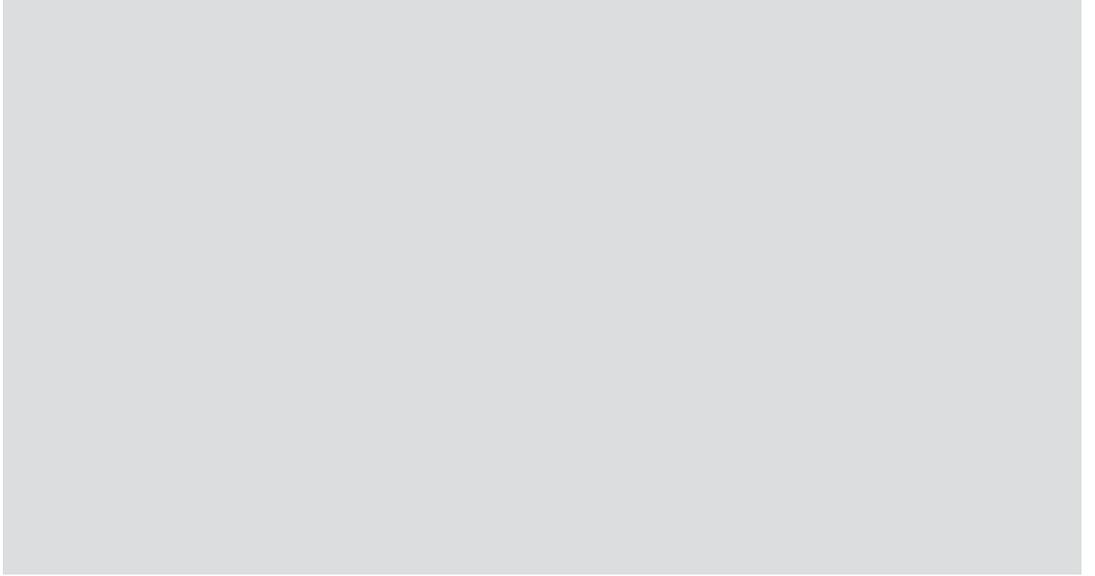


## LIST OF ABBREVIATIONS

[ <sup>123</sup> I]ADAM	<sup>123</sup> iodine-labeled 2-([2-({dimethylamino}methyl)phenyl]thio)-5-iodophenylamine
[ <sup>123</sup> I]β-CIT	<sup>123</sup> iodine-labeled 2β-carbomethoxy-3β(4-iodophenyl)tropane
[ <sup>11</sup> C]DASB	[ <sup>11</sup> C]amino-4-(2-dimethylaminomethylphenylsulfanyl)benzonitrile
[ <sup>11</sup> C]McN5652	Trans-1,2,3,5,6,10-β-hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-∇] isoquinoline
<sup>1</sup> H-MRS	Proton Magnetic Resonance Spectroscopy
5-HIAA	5-hydroxyindoleacetic acid
5-HT	Serotonin
5-HTTLPR	5-HT Transporter Promoter Gene Region
ADC	Apparent Diffusion Coefficient
AIR	Automated Image Registration Library
ANOVA	Analysis of Variance
APP	AMC Postprocessing Package
BDI	Beck Depression Inventory
BIS	Barratt Impulsivity Scale
BOLD	Blood Oxygen Level Dependent
BS	Boredom Susceptibility
CBCL	The Child Behavior Checklist
CBF	Cerebral Blood Flow
CBV	Cerebral Blood Volume
CD	Cumulative Dose
BET	Brain Extraction Tool
Cho	Choline-containing compounds
CI	Confidence Interval
CIDI	Composite International Diagnostic Interview
COWAT	Controlled Oral Word Association Test
Cr	Creatine plus phosphocreatine
CSF	Cerebrospinal Fluid
DART	Dutch Adult Reading Test
DAT	Dopamine Transporter
DIS	Disinhibition
DSC	Dynamic Susceptibility Contrast
DOPAC	3,4- dihydroxyphenylacetic acid
DSM	Diagnostic and Statistical Manual
DTI	Diffusion Tensor Imaging
DV	Distribution Volumes
DWI	Diffusion Weighted Imaging

ES	Experience Seeking
EPI	Echo Planar Imaging
FA	Fractional Anisotropy
FAST	fMRIB's Automated Segmentation Tool
FDA	Food and Drug Administration
FLIRT	Fast Linear Registration Tool
fMRI	functional Magnetic Resonance Imaging
FSL	fMRIB Software Library
FOV	Field of View
FSPGR	Fast Spoiled Gradient Echo
FWHM	Full-Width at Half-Maximum
i.m.	Intramuscular
JOLO	Judgement of Line Orientation
LCModel	Linear Combination of Model spectra
LR	Likelihood Ratio
MANOVA	Multivariate Analysis of Variance
MDEA	3,4-methylenedioxy- <i>N</i> -ethylamphetamine
MDA	3,4-methylenedioxyamphetamine
MDMA	3,4-methylenedioxymethamphetamine
mI	Myo-Inositol
MNI152	Montreal Neurological Institute brain template
MRI	Magnetic Resonance Imaging
NAA	N-acetylaspartate
NART	National Adult Reading Test
NeXT study	Netherlands XTC Toxicity study
OR	Odds Ratio
PASAT	Paced Auditory Serial Addition Test
PD	Proton Density
PET	Positron Emission Tomography
p.i.	Post-Injection
PWI	Perfusion Weighted Imaging
RAVLT	Rey Auditory Verbal Learning Test
ROI	Region of Interest
rrCBV	regional relative Cerebral Blood Volume
SBL	Spanning Behoefte Lijst (sensation seeking scale)
s.c.	Subcutaneously
S.D.	Standard Deviation
S.E.M.	Standard Error of the Mean
SERT	Serotonin Transporter
SPECT	Single Photon Emission Computed Tomography

SSRI	Selective Serotonin Reuptake Inhibitor
TAS	Thrill and Adventure Seeking
TE	Echo Time
TR	Relaxation Time
XTC	Ecstasy
WAIS-R	Wechsler Adult Intelligence Scale- revised
WCST	Wisconsin Card Sorting Test
WMS-R	Wechsler Memory Scale – Revised



## List of Publications



## LIST OF PUBLICATIONS

**De Win MML**, Theuvsen WJ, Roche PW, de Bie RA, van Mameren H. The paper grip test for screening on intrinsic muscle paralysis in the foot of leprosy patients. *Int J Lepr Other Mycobact Dis.* 2002; 70: 16-24.

Booij J, de Bruin K, **de Win MML**, Lavini C, den Heeten GJ, Habraken JB. Imaging of striatal dopamine transporters in rat brain with single pinhole SPECT and co-aligned MRI is highly reproducible. *Nucl Med Biol.* 2003; 30: 643-649.

**De Win MML**, Reneman L, Reitsma JB, den Heeten GJ, Booij J, van den Brink W. Mood disorders and serotonin transporter density in ecstasy users—the influence of long-term abstinence, dose, and gender. *Psychopharmacology (Berl).* 2004; 173: 376-382.

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**De Win MML**, Jager G, Booij J, Reneman L, Schilt T, Lavini C, Olabbarriaga SD, Ramsey NF, den Heeten GJ, van den Brink W. Converging evidence for specific toxic effects of ecstasy on the thalamus using MR and SPECT imaging. Submitted.

**De Win MML**, Jager G, Booij J, Reneman L, Schilt T, Lavini C, Olabbarriaga SD, den Heeten GJ, van den Brink W. Sustained effects of ecstasy on the human brain: a prospective neuroimaging study in novel users. Submitted.

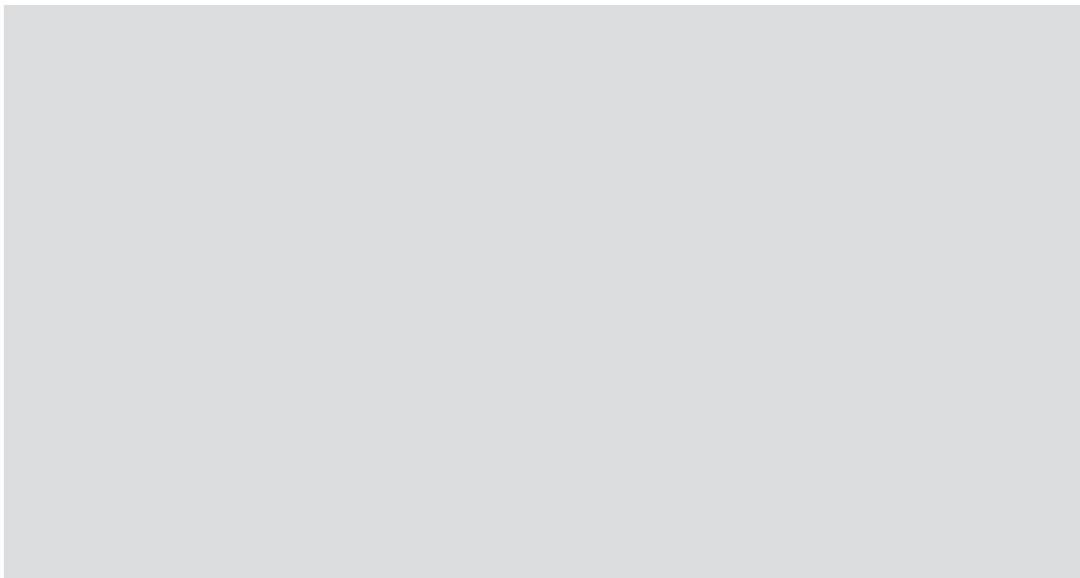
Jager G, **de Win MML**, van der Tweel I, Schilt T, Kahn RS, van den Brink W, van Ree JM, Ramsey NF. Sustained effects of ecstasy on neurocognitive brain function in the context of poly-substance use in humans. Submitted.

Jager G, **de Win MML**, Vervaeke HKE, Schilt T, Kahn RS, van den Brink W, Jan M. van Ree J, Ramsey NF. Incidental use of Ecstasy: no evidence for harmful effects on cognitive brain function in a prospective fMRI study. Submitted.

Schilt T, **de Win MML**, Jager G, Koeter M, Schmand B, van den Brink W. Estimating the specific effects of ecstasy and other illicit drugs on cognition in a sample of poly-substance ecstasy users. Submitted.

Reneman L, **de Win MML**, den Heeten GJ, Booij J, van den Brink W. Exposure to high levels of serotonin during brain development leads to enhanced outgrowth of the serotonergic system: initial observations with users of ecstasy (MDMA). Submitted.





# Dankwoord



## DANKWOORD

In juli 2001 ben ik als arts-onderzoeker begonnen aan de ambitieuze NeXT (Netherlands XTC Toxicity) studie en nu na ruim 5½ jaar ligt hier het resultaat, mijn proefschrift. De NeXT studie is tot stand gekomen en geslaagd dank zij een intensieve en harmonieuze samenwerking van de afdelingen Radiologie, Nucleaire Geneeskunde, Psychiatrie en Verslavingszorg en Medische Informatica van het Academisch Medisch Centrum, het Criminologisch Instituut Bongers en het Science Center van de Universiteit van Amsterdam, de afdeling Psychiatrie van het Universitair Medisch Centrum Utrecht en de afdeling Kinder- en Jeugdpsychiatrie van het Sophia Kinderziekenhuis, Erasmus Medisch Centrum.

Ik realiseer me dat het nooit zover was gekomen zonder al die vele mensen die op de een of andere manier hun bijdrage hebben geleverd en ik wil ze dan ook enorm hartelijk danken. Daarnaast wil ik hier enkele personen in het bijzonder bedanken:

Allereerst gaat mijn dank uit naar de ruim 300 **vrijwilligers** van dit onderzoek, die onze tijdrovende en vrij ingrijpende onderzoeken ondergingen en zonder wie dit onderzoek niet mogelijk was.

Daarnaast natuurlijk mijn dank voor de directe begeleiders van dit onderzoek, met wie ik zeer prettig heb samengewerkt en alle kansen heb gekregen om zelfstandig onderzoek te doen.

Mijn promotoren, **prof. dr. W. van den Brink** en **prof. dr. G. J. den Heeten**:

Beste **Wim**, jij bent de belangrijkste initiator van de NeXT studie en ik heb er bewondering voor dat je deze zeer ambitieuze studie hebt aangedurfd en dat je er altijd vertrouwen in hebt gehad dat het goed zou aflopen. Jij was degene die de boel bij elkaar hield en ik vind het erg bijzonder dat je (naast al je andere projecten) met je helikopter view steeds het overzicht hield en overal van op de hoogte was. Hartelijk dank voor je enthousiasme en zeer waardevolle en snelle commentaar op mijn manuscripten.

Beste **Ard**, hartelijk dank voor je enthousiasme voor natuurlijk vooral het imaging gedeelte van dit onderzoek en grenzeloze vertrouwen in mijn werkzaamheden als onderzoeker. Ik hoop dat jouw enthousiasme voor het imaging onderzoek naar neuropsychiatrische aandoeningen en de door jouw geïnitieerde en onontbeerlijke samenwerking met de beeldbewerkers behouden zullen blijven voor de afdeling. Het zou daarbij erg leuk zijn als er nog voortgeborduurd gaat worden op de spin-off van de NeXT studie.

Mijn co-promotoren, **dr. L. Reneman** en **dr. J. Booij**:

Beste **Liesbeth**, jij bent mijn voorganger in het ecstasy onderzoek en hebt een groot aandeel gehad in de opzet van het huidige project. Door je drukke opleiding heb je de afgelopen jaren wat minder tijd kunnen besteden aan de wetenschap, maar het ziet ernaar uit dat dat in de nabije toekomst weer goed gaat komen. Ondanks je drukke bezigheden zag je altijd kans om in je vrije tijd zeer gedegen en enthousiast (soms wist je van data die leken op ‘niets’ toch nog ‘iets’ te maken) commentaar te geven op mijn studievoorstellen, METC aanvragen en manuscripten, waarvoor ik je heel hartelijk wil danken.

Beste **Jan**, jij was degene die altijd beschikbaar was voor raad, daad en een praatje. Hartelijke dank voor je goede ideeën voor substudies, discussie paragrafen en je wetenschappelijke inzicht. Ik hoop dat we de komende jaren nog verder zullen samenwerken aan de spin-off van de NeXT studie en misschien ook wel aan nieuwe methoden om de SERT nog beter te kunnen imageren.

De leden van de promotie-commissie:

**Prof. dr. J. S. Laméris**, hartelijk dank dat ik de gelegenheid heb gekregen eerst als onderzoeker en nu als arts-assistent op ‘uw’ afdeling Radiologie te mogen werken. Ik heb het werkklimaat en afdelingssfeer altijd als zeer prettig ervaren. Hartelijk dank ook dat u zich als commissielid, in dit voor u waarschijnlijk niet zo voor de hand liggende onderwerp, wilde verdiepen.

**Prof. dr. B. L. F. van Eck-Smit**, hartelijk dank dat ik op de woensdagen alle gelegenheid kreeg op de afdeling Nucleaire Geneeskunde mijn onderzoek uit te voeren, je belangstelling in het verloop van het onderzoek en natuurlijk voor het zitting nemen in de promotiecommissie.

**Prof. dr. D. J. Veltman**, hartelijk dank voor de geweldige hulp bij de soms behoorlijk ingewikkelde SPM analyses van de SPECT data en dat u commissielid wilde zijn.

De overige leden **prof. dr. R. S. Kahn**, **prof. dr. M. A. van Buchem** en **dr. R. J. Verkes**, wil ik ook hartelijk danken voor hun bereidheid zitting te nemen in de promotiecommissie.

De ‘meisjes’ van de NeXT studie, **Gerry Jager**, **Hylke Vervaeke** en **Thelma Schilt**:

Ondanks dat de studie voor ons soms met ups en downs verliep hebben we het er toch maar goed vanaf gebracht en ik wil jullie danken voor de fijne samenwerking. **Gerry**, ontelbare mailtjes zijn over en weer gegaan de afgelopen jaren. Ondanks dat we ieder ons eigen onderzoeksdeel hadden moest er veel overlegd worden en ik ben blij dat dat zo goed is gegaan. Veel dank ben ik je met name verschuldigd voor de ‘XTC+ studie’, dat begon als een klein uitstapje, maar die mede dankzij jouw duidelijke visie, rekruteringsstrategie, en statistische hoogstandjes een hele mooi studie is geworden.

**Hylke**, jij hebt waarschijnlijk het moeilijkste en meest frustrerende deel van de studie voor je rekening genomen, het rekruteren van bijna 200 mensen die waarschijnlijk in de nabije toekomst voor het eerst ecstasy zouden gaan gebruiken en die ook nog eens moesten voldoen aan al onze criteria. Ik heb veel bewondering voor je aanpak en doorzettingsvermogen en het is toch wel erg bijzonder dat jullie achteraf gezien ook echt de goede groep te pakken hadden. **Thelma**, hartelijk dank voor je nauwgezette afname van alle neuropsychologische onderzoeken (432 x 1½ uur) op de vaak behoorlijk hectische woensdagen. Het schema was door mij zo in elkaar gepast dat je regelmatig 7½ uur achter elkaar aan het testen was zonder pauze, maar ondanks dat ben je in de loop van de tijd steeds enthousiaster geworden voor de ‘wetenschap’ en ik vind het dan ook erg leuk dat jij ondertussen met al deze bijzondere data ook aan een promotietraject bent begonnen. Je eerste artikel was meteen een klapper, heel veel succes met het vervolg!

**Dr. Nick F. Ramsey** en **dr. Dirk J. Korf** wil ik hartelijk danken voor hun bijdrage als mede-initiatoren van de NeXT studie en voor de nuttige discussies over studie opzet, vorderingen en manuscripten tijdens de NeXT vergaderingen. Ook **prof. dr. Ben Schmand** wil ik bedanken voor zijn bijdrage aan deze discussies.

De postprocessing en analyses van alle 408 (x3) <sup>1</sup>H-MRS, 416 perfusie en 413 diffusie gewogen MRIs en 365 4h en 159 24h SPECT scans bleek behoorlijk ingewikkeld en dit zou nooit gelukt zijn zonder de hulp van zeer veel mensen die hierbij betrokken zijn geweest. Ik wil alle fysici, ‘smartguy’s’ en ‘computerwhizzkids’ heel hartelijk danken dat jullie mij hierbij geholpen hebben en dat veel van jullie er ondanks jullie eigen werkzaamheden veel meer tijd hebben ingestoken dan eigenlijk gepland was. **Dr. C. Lavini**, beste **Cristina**, hartelijk dank voor je ondersteuning bij het opzetten van de MRI protocollen en bij de analyse van de spectroscopie data. **Dr. E.J.P. Vlieger**, beste **Erik-Jan**, hartelijk dank voor je pragmatische instelling, snelle werkwijze en ingenieuze opzet van de automatische DTI en PWI analyses die mij erg veel werk hebben bespaard. **Dr. S. D. Olabariaga**, beste **Silvia**, je kwam als een reddende engel, toen het automatische systeem toch nog niet helemaal waterdicht bleek te zijn en ik bijna de moed had opgegeven dat het ooit nog goed zou komen. Terwijl je eigenlijk alleen onze studie als testcase zou gebruiken voor het virtuele lab was je zeer snel in staat het ‘erikjaniaans’ te ontcijferen, kritisch te bekijken en te optimaliseren zodat we uiteindelijke betrouwbare data hebben gekregen. **Dr. J. Snel**, beste **Jeroen**, bedankt voor je hulp bij ingewikkelde computerzaken, met name nadat mijn linux computer totaal gecrashed was en alles weer opnieuw moest worden opgezet. **Prof. dr. M van Herk**, beste **Marcel**, ik ben zeer onder de indruk van wat jij allemaal kan met ‘plaatjes’. Heel hartelijk dank voor je hulp bij het matchen van de SPECT en MRI

scans. Voor de analyses van de SPECT scans ben ik daarnaast veel dank verschuldigd aan **dr. Frans Vos, ir. Jan Habraken en Matthan Caan**.

‘Mijn’ onderzoeksassistenten/studenten: **Ivo Bisschops** en **Sarah Dijkink**, zonder jullie zouden de woensdagen totaal in de soep gelopen zijn en ik ben blij dat jullie er drie jaar lang elke woensdag waren om de inclusie en de logistiek van de studie te ondersteunen. **Laurens Schrama**, bedankt voor je hulp bij de analyses van het SERT gen polymorfisme; **Rogier de Jeu**, bedankt voor je bijdrage aan de ‘ratten studie’ en **Benoit Faivre**, merci beaucoup pour le postprocessing et les analyses des SPECT scans.

De **laboranten van de afdeling Nucleaire Geneeskunde**, hartelijk dank voor het maken van alle SPECT scans. In het bijzonder wil ik hiervoor **Jacco Visser** danken die hiervan het grootste deel voor zijn rekening heeft genomen en drie jaar lang bereid was op de woensdagavonden bij te klussen.

**Onno van Eijkelenburg, Jean-Paul Geerets, Martin Schrijnders en Jan Wolters**, hartelijk dank voor de computer ondersteuning.

Hoewel de data van het Zuid-Holland cohort nog niet in dit proefschrift verwerkt zijn wil ik **prof. dr. F. C. Verhulst** bedanken voor het beschikbaar stellen van ‘zijn’ cohort en **dr. Jan van der Ende** hartelijk danken voor het aanleveren van alle benodigde data en de samenwerking.

Zonder mijn collega onderzoekers van de afdeling Radiologie was mijn onderzoekstijd lang niet zo leuk en leerzaam geweest. Bij hen kon ik altijd terecht voor de lunch, voor een luisterend oor als het een keer tegengat, voor de bespreking van het weekend, voor een wandeling naar de koffieautomaat, voor het uitwisselen van onderzoekstactieken of gewoon voor de gezelligheid (leuk dat jullie naast het werk ook mee wilden naar wijncursus, bedrijfshockey, golfles, kunstgeschiedenis en schilderles). **Maaïke** en **Nicole**, jullie waren geweldig als mede-powerpuff girls en kamergenoten, super om nu ook samen in opleiding te zijn! **Jasper, Karin, Adrienne, Annette, Rogier, Ayso, Shandra, Sebastiaan, Wouter** en **Anneke**, enorm bedankt.

**Stafleden en collega-assistenten** van de afdeling Radiologie van het AMC, bedankt voor jullie interesse en steun. Dank zij jullie is de overgang van onderzoeker naar assistent me heel goed bevallen.

Lieve **familie, vrienden** en **Topaasjes**, jullie vriendschap is me enorm dierbaar. Bedankt voor jullie regelmatige afleiding, belangstelling en steun tijdens mijn

onderzoeksperiode en in het bijzonder tijdens de afgelopen af en toe moeilijke tijd. Ik hoop de komende periode weer wat achterstallige afspraken in te halen.

Mijn schoonfamilie wil ik hartelijk danken voor hun warme belangstelling voor ons reilen en zeilen en voor de vorderingen van mijn onderzoek. Lieve **Marina** en **Jankees**, heerlijk om regelmatig in hectische tijden bij jullie in Spanje tot rust te komen. Lieve **Thijs** en **Claartje**, ik vind het leuk dat ik toch nog een beetje de artsentradiatie in de familie kan voortzetten (en wie weet gaat de kleine Paré zijn voorvaderen weer achterna). Lieve **Willemijn** en **Toini**, nu ik klaar ben met mijn 'grote project' kom ik echt snel een keer naar Istanbul!

Mijn beide paranimfen, ik vind het geweldig dat jullie op de grote dag naast me zullen staan.

Lieve **Marthe**, vriendinnetje sinds het eerste college in Maastricht. Ik vind het super dat je me de 2<sup>e</sup> (ook met dikke buik) zult steunen. Ik verheug me al op ons gezamenlijke verlof. Lieve **Pol**, ik ben er enorm trots op dat jij mijn broertje en maatje bent! Ik vind onze hechte band heel speciaal. Je neefje boft straks maar met zo'n (suiker)oom.

Lieve **papa** en **mama**, ik bof maar enorm met jullie als ouders en heb daarom dit 'levenswerkje' aan jullie opgedragen. Ontzettend bedankt voor alle mogelijkheden, onvoorwaardelijke liefde, vertrouwen en steun die jullie mij (ons) gegeven hebben en nog steeds geven. Papa, ik ben er trots op om in hetzelfde vakgebied te werken als jij en ik waardeer het enorm dat je je (waarschijnlijk als een van de weinige) door mijn hele proefschrift hebt geworsteld om me te helpen de puntjes op de i te zetten.

Lieve **Adriaan**, mijn grote liefde. Jij laat je werkelijk door niets uit het veld slaan en ziet overal mogelijkheden en oplossingen, dat bewonder ik enorm in je. De afgelopen hectische maanden hebben mij weer eens doen beseffen dat ik hierdoor samen met jou de hele wereld aan kan. Dank je wel voor je liefde en steun, ook was dat promoveren voor jou niet altijd even gezellig. Ik ben ervan overtuigd dat je een fantastische papa zal zijn!



## CURRICULUM VITAE



Maartje Maria Léontien de Win was born on Ascension Day on May 27th 1976 in Eindhoven. She grew up most of her youth in Apeldoorn and completed 'VWO' at the 'Stedelijk Gymnasium Apeldoorn' in 1994. At the age of eighteen she moved to Belgium to start her medical studies at the University of Antwerp (RUCA). After she passed her first year with honour, she continued her studies in The Netherlands at the Maastricht University. During her studies she had her first experience with scientific research during a 4-month research project at The Purulian Leprosy Home and Hospital, Purulia, West-Bengal, India on intrinsic muscle paralysis in the foot of leprosy patients. She passed for her MSc in January 1999 and for her MD in October 2000 (both *cum laude*).

After four months of backpacking from Singapore to Beijing through 7 different countries in Asia, she started her PhD career in July 2001 as 'arts onderzoeker' on the Neurotoxicity of XTC (NeXT) study, described in this thesis, at the department of Radiology of the Academic Medical Center, University of Amsterdam. She worked under supervision of prof. dr. W. van den Brink (department of Psychiatry) and prof. dr. G.J. den Heeten (department of Radiology) in close collaboration with the department of Nuclear Medicine of the Academic Medical Center, The Bongor Institute of the University of Amsterdam and the department of Psychiatry of the University Medical Center Utrecht. During this research period she was PhD student of the Graduate School Neurosciences Amsterdam and presented her results at national and international congresses. In December 2005 she started her radiological training as a resident at the Academic Medical Center, University of Amsterdam (dr. O.M. van Delden and prof. dr. J.S. Laméris).

Maartje lives together with Adriaan Paré and they expect their first child at the end of March 2007.