Investigating the potential neurotoxicity of ecstasy (MDMA). An imaging approach
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An ecstasy tablet is unlike pretty much every other illicit drug. It produces a sensation of euphoria that one can’t quite get anywhere else. “It gives me this great pleasure. All my worries seem to go away. I don’t think of what was or will be. I am just enjoying the present moment” says Vlata River (TIME Magazine 2000). “I couldn’t dance all night without it. I would get tired soon”. It is not physically addictive, teenagers everywhere have begun experimenting with it.

The evolution of ecstasy, from an underground urban drug to an almost mundane feature of middle-class life in the U.S. and Europe, peaked in popularity in the early 1990s, when raves began to infiltrate the nightlife. In the U.S., ecstasy remained common only in several subcultures, but in the past few years, it has gone mainstream both in U.S. and Europe. Drug laboratories, most of which have their origins in the Netherlands and Eastern Europe, have taken over the trade. Currently around 5% of young adults in Europe say they have tried ecstasy. In a survey of illicit drug use amongst British university students 13% stated that they had taken ecstasy at least once, while 3% used it regularly (Webb et al., 1996). In 1999, 5% of the Dutch pupils (14 and 15 years old) indicated having tried ecstasy (source: NDM Annual report 2000). Several studies suggest that the recreational use of the drug seems to be increasing in every westernized country studied (Schifano et al., 1998; Parrott et al., 2000; NIDA, 1997). Ecstasy is popular because one experiences few negative consequences. On the surface it appears to be a safer drug than alcohol and cocaine, at least in the short run. However, this perceived safety is at odds with evidence from animal studies indicating that the ring-substituted amphetamine derivative and active component of ecstasy tablets, (+)3,4-methylenedioxymethamphetamine (MDMA), produces toxic effects on brain serotonin (5-HT) neurons (for review see Ricaurte et al., 2000). This raises the question of whether MDMA is also neurotoxic in humans.

This thesis focuses on the potential neurotoxic effects of MDMA, although problems associated with pills adulterated with other substances and potential neurotoxicity of other amphetamine derivatives will also be discussed. In this introduction, the background of MDMA’s neurochemistry and neurotoxicity is given, and aims and outline of the thesis are discussed.

Neurochemistry

MDMA belongs to a group of ring-substituted amphetamine derivatives which bear structural and pharmacological similarities to both the psychomotor stimulantamphetamine and hallucinogens such as mescaline (Steele et al., 1994).

MDMA is a potent monoaminergic agonist which both inhibits the reuptake and promotes the release of serotonin (5-HT) (Steele et al., 1987) and, to a lesser extent of dopamine (Hiramatsu et al., 1990; Yamamoto et al., 1988). The stereochemical profile for MDMA’s neurochemical effects is similar to related amphetamines in which the S- (+)-MDMA is more active than R- (-)-MDMA (Johnson et al., 1986; Steele et al., 1987).

It is presently unclear whether the psychoactive, as opposed to the neurotoxic, effects of MDMA result from the pre-synaptic release of 5-HT, or from interactions with post-synaptic target receptors, particularly 5-HT receptors (Mckenna et al., 1990). Radioligand binding assays have shown that MDMA has highest affinity for the 5-HT transporter (K = 0.6 μM), followed by 5-HT1A receptors (1.5 μM), the α2-adrenergic receptor (3.6 μM), the histamine H1 receptor (5.7 μM), and the muscarinic M1 receptor (5.8 μM). MDMA has no clear affinity for dopamine D1 or D2 receptor sites (Battaglia et al., 1988a; Mckenna & Peroutka 1990).

In vivo metabolic pathways of MDMA in the rat include N-demethylation, O-dealkylation, deamination and conjugation (Lim et al., 1988). The O-dealkylated catechol metabolite appears only in the brain, is mediated by cytochrome P450 isozymes and is a primary route of metabolism in rats (Lin et al., 1992). In vivo stereochemical studies have suggested a more rapid and extensive metabolism of the S- (+)-MDMA (Cho et al., 1990; Fitzgerald et al., 1990). Half life estimates in rats for the enantiomers are 73.8 and 100.7 min for S- (+) and R- (-) MDMA, respectively (Cho et al., 1990).

Neurotoxicity

More than a decade ago the first evidence emerged indicating that MDMA produced selective toxic effects on brain 5-HT neurons (Ricaurte et al., 1985). Dose-related reductions in brain markers of 5-HT axons, such as S-5-HT and 5-hydroxyindolacetic acid (5-HIAA) (Commins et al., 1987; Schmidt et al., 1987a; 1986; Stone et al., 1986), the density of 5-HT transporters (Battaglia et al., 1987; Commins et al., 1987), the density of vesicular monoamine transporters (Ricaurte et al., 2000), and the activity of tryptophan hydroxylase (Schmidt et al., 1987b; Stone 1985) have consistently been reported in animals treated with MDMA. These
neurochemical deficits, which last well beyond the period of drug administration, have been correlated with the disappearance of 5-HT immunoreactive axons (Molliver et al., 1990; O’Hearn et al., 1988; Wilson et al., 1989) and have been confirmed using silver impregnation of degenerating neurons (Commins et al., 1987).

Neurotoxic effects of MDMA on 5-HT neurons have been demonstrated in a variety of animal species. The magnitude and duration of MDMA’s effects are dependent upon the dose and number of injections given (Steele et al., 1994). Single doses of 10 mg/kg have been shown to produce marked transient depletions in 5-HT and 5-HIAA in rat brain persisting for one week or longer (O’Shea et al., 1998). Primates are much more vulnerable to the neurotoxic actions of MDMA than rodents. A single dose of 5 mg/kg MDMA has been shown to produce long-lasting depletion of 5-HT in monkey brain (Ricaurte et al., 1988). Brain levels of dopamine and its metabolite are not reduced by low doses of MDMA, but are depleted after higher doses (Commins et al., 1987), suggesting that while MDMA is more toxic to 5-HT than dopaminergic systems, it can also damage dopaminergic neurons.

There is evidence indicating that 5-HT-rich brain regions differ in their sensitivity to MDMA neurotoxic effects. Areas rich in 5-HT terminals such as the cerebral cortex show more severe deficits than brain regions containing fibers of passage (hypothalamus) or cell bodies (brain stem) (Commins et al., 1987; O’Hearn et al., 1988). Some evidence exists that the 5-HT system of rats is able to recover within six months to one year following repeated injections with 10 or 20 mg/kg MDMA (Battaglia et al., 1988b; Scanzello et al., 1993). In non-human primates the neurotoxic effects of MDMA may be permanent in some (particularly cortical) brain regions (Hatzidimitriou et al., 1999; Ricaurte et al., 1992a; Ricaurte et al., 1992b), while other brain regions (hypothalamus and thalamus) show evidence of complete recovery. It has been suggested that the distance of the affected axon terminal field from the rostral raphe nuclei influences recovery of 5-HT axons after MDMA injury (Hatzidimitriou et al., 1999).

These observations in animals may be relevant to humans, since doses used by humans fall squarely into the range of dosages that are toxic in animals, when dosages are adjusted to account for interspecies differences (Ricaurte et al., 2000). The observation that smaller animal species require higher doses of drug to achieve equivalent drug effects is predicted by the principle of interspecies scaling. This method utilizes known relations between body mass/surface area and accounts for differences in drug clearance (Mordenti & Chappell 1989). Using this method, the equivalent known neurotoxic dose of MDMA in rats (20 mg/kg, body weight) is found to be 5 mg/kg in monkeys, which dose, indeed, has been shown to be neurotoxic. Similarly, using the same technique, it is possible to predict dosages of MDMA that would be neurotoxic in humans, based on those that are neurotoxic for rats or monkeys. The equivalent dose in humans of 5 mg/kg in a 1 kg squirrel monkey is found to be 1.28 mg/kg or approximately 96 mg for a 75 kg individual (McCann et al., 2001). Human MDMA users typically use single doses of MDMA of 75-125 mg. As such, animal models of MDMA-induced neurotoxicity suggest that human MDMA users might be at high risk for incurring 5-HT neurotoxicity.

If MDMA damages serotonergic circuits in humans, what functional consequences could be expected? 5-HT is thought to play a role in regulating cognitive function, mood, anxiety, impulsivity, sleep and appetite. Therefore, 5-HT neurotoxic lesions in humans may induce important ramifications for the mental health and psychological function of people who use this drug. For instance, 5-HT imbalance has been postulated to underlie psychiatric disorders including mainly depression, anxiety, panic disorder, and disorders of impulse control. Sleep and personality studies have demonstrated altered sleep architecture and increased impulsivity in MDMA users (Allen et al., 1993; Gerra et al., 1998; Morgan 1998). In addition, there have been many case reports of neuropsychiatric sequelae after MDMA use, including depression, psychotic disorders, panic disorders, social phobia and bulimic episodes (for review see Schifano et al., 2000). Finally, a number of studies have evaluated cognitive function in MDMA users as an indicator of MDMA-induced 5-HT functional impairment. The bulk of these studies found deficits in verbal and visual memory in MDMA users using neuropsychological testing (for review see Parrott et al., 2000).

Aims of the thesis
In view of MDMA’s popularity as a recreational drug, animal studies demonstrating serotonergic degeneration after MDMA administration at doses that overlap those used by humans, and the role 5-HT plays in several essential functions, it is important to determine whether MDMA is neurotoxic to 5-HT neurons in humans.

In contrast to the numerous animal studies, the
number of studies investigating the neurotoxic potential in human MDMA users is limited. This is probably because previously no methods were available for directly evaluating the neurotoxic effects of MDMA in living humans. Studies of MDMA's neurotoxic potential in humans had to rely on indirect methods, including measurements of 5-HIAA in cerebrospinal fluid (CSF), and neuroendocrine challenge techniques. However, recent development of in vivo neuroimaging tools, such as positron emission tomography (PET), single photon emission computed tomography (SPECT), and several magnetic resonance (MR) imaging applications are able to directly provide insights into the effects of MDMA on the living human brain. Furthermore, these imaging-techniques have the ability to identify several potential functional consequences of MDMA-induced neurotoxicity, and may be useful in identifying unknown but potential long-term effects.

Therefore, the present study was designed in order to investigate more directly, with the use of SPECT and MRI, the potential neurotoxicity of MDMA in the human brain, and to study potential functional consequences hereof.

In this thesis the following questions are addressed:

1. Can MDMA-induced 5-HT neurotoxicity be studied using SPECT?
   Hypothesis: $^{[12]}$CIT-SPECT is a useful method in detecting MDMA-induced 5-HT neuronal loss.

2. Are heavy and moderate MDMA users at risk of developing (5-HT) brain pathology?
   Hypothesis: MDMA users demonstrate a dose-related reduction in biological markers of (5-HT) neuronal loss.
   Sub 2.1. If MDMA users are at risk of developing (5-HT) brain pathology, are there gender differences in the susceptibility of the neurotoxic effects of MDMA?
   Hypothesis: males and females differ in their susceptibility to MDMA's neurotoxic effects.
   Sub 2.2. If MDMA users are at risk of developing (5-HT) brain pathology, what are the effects of long term abstinence?
   Hypothesis: In ex-MDMA users, biological markers of (5-HT) neuronal loss are reduced in some brain regions, while at control levels in other brain regions.

3. Are MDMA users at risk of developing dopamine brain pathology?
   Hypothesis: Use of MDMA does not reduce dopamine transporter densities.

4. If MDMA use leads to 5-HT brain pathology, this may result in impairments of functions in which 5-HT plays an important role. Thus, the following question was examined: is the use of MDMA associated with 5-HT functional impairments as evidenced by alterations in: (1) post-synaptic 5-HT, receptor densities, (2) brain microvasculature, and (3) cognitive functioning?
   Hypothesis: MDMA use alters markers of 5-HT function.

5. If indeed MDMA use leads to impairments in functions in which 5-HT has been implicated, are these associated with MDMA-induced neuronal loss?
   Hypothesis: memory impairments in MDMA users are associated with a reductions in biological markers of (5-HT) neuronal loss.

Outline of the thesis

This thesis has five parts. In Part I a general introduction and outline of the thesis is given. Part II reports on the potential neurotoxicity of MDMA in the human brain, part III studies potential functional consequences of MDMA-induced neuronal loss, part IV studies whether biological markers associated with MDMA-induced neuronal loss are correlated with memory function, and part V gives a summary and conclusion of the thesis.

Part I: Introduction

A general introduction and outline of the thesis is given.

Part II: Biological markers of neuronal loss

In part II different techniques and (by inference) biological markers are studied to investigate whether MDMA use is associated with neuronal loss in the human brain.

Chapter 1 includes the general introduction and outline of the thesis. Chapter 2 describes a set of experiments in rats and monkey brain to identify and validate the radioligand best suited to study MDMA-induced loss of 5-HT neurons with SPECT. In Chapter 3, the effects of MDMA on the human brain are described by quantification of brain 5-HT transporter densities using $^{[12]}$CIT-SPECT, in different groups of MDMA users and control subjects. In Chapter 4 a study is described in which the effects of MDMA use on (non-specific) neurons are studied by measuring N-acetylaspartate levels in several brain regions using proton MR Spectroscopy (H MRS). Chapter 5 reports on the effects of ecstasy on DA neurons in the human brain by quantification of brain DA transporter dens
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ties using [123]β-CIT SPECT. In addition, the potential neurotoxicity of another amphetamine derivative, methylphenidate, on 5-HT and DA transporter densities is studied in rat brain.

Part III: Potential functional consequences of MDMA-induced neuronal loss
Part III of this thesis investigates whether the use of MDMA is associated with impairments of functions in which 5-HT is thought to play an important role.

Since 5-HT has been shown to regulate postsynaptic 5-HT, receptor densities, chapter 6 investigates the relation between regional 5-HT levels and 5-HT receptor densities in rat brain. In parallel, 5-HT receptor densities in the cerebral cortex of recent as well as ex-MDMA users were studied using [111]R91150 SPECT. Since 5-HT receptors play a role in the regulation of brain microvasculature, chapter 7 discusses whether changes in brain 5-HT receptor densities are associated with alterations in blood vessel volumes (rCBV) using [111]R91150 and perfusion MR imaging, respectively. In addition, the diffusional motion of water molecules, a potential marker for tissue changes in degenerating brain tissue, is studied in MDMA users using diffusion weighted MR imaging. Chapter 8 reports on the effect of MDMA on cognitive function, since 5-HT has been shown to play an important role in cognitive functioning. Different groups of MDMA users and control subjects were studied using a neuropsychological test battery that specifically relates to serotonergic functions, particularly memory.

Part IV: Linking biological markers of neuronal loss with memory function
To further investigate whether the functional impairments described in part II of this thesis are indeed caused by MDMA use, the association between different biological markers of neuronal loss and memory function is discussed in chapter 9 of this thesis.

Part V: Summary and conclusion
Chapter 10 gives an overview of the contributions that in vivo brain imaging tools have made to our understanding of the neurotoxic effects and functional consequences of MDMA use, thereby bringing most of the studies presented in this thesis in perspective with the international literature. Chapter 11 discusses the outcome of this thesis and the implications thereof.

References
Allen RP, McCann UD, Ricaurte GA. Persistent effects of (+/-)3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") on human sleep. Sleep 1993; 16:560-564.
Battaglia G, Brooks BP, Kulsakdinun C, De Souza EB. Pharmacological profile of MDMA (3,4-methylenedioxymethamphetamine) at various brain recognition sites. Eur J Pharmacol 1988a; 149:159-165.


McKenna DJ, Peroulaika SJ. Neurochemistry and neurotoxicity of 3,4-methylenedioxy-methamphetamine (MDMA, "ecstasy"). J Neurochem 1990; 54: 14-22.


Morgan MJ. Recreational use of "ecstasy" (MDMA) is associated with elevated impulsivity. Neuropsychopharmacology 1998; 19: 252-264.


