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Neuroimaging findings with MDMA/ecstasy: technical aspects, conceptual issues and future prospects

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Abstract

Users of ecstasy (3,4-methylenedioxymethamphetamine; MDMA) may be at risk of developing MDMA-induced injury to the serotonin (5-HT) system. Previously, there were no methods available for directly evaluating the neurotoxic effects of MDMA 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. Single photon emission computed tomography (SPECT), positron emission computed tomography (PET) and proton magnetic resonance spectroscopy (^{1}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. 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 5-HT neurotoxicity. There seem to be dose-dependent and transient reductions in SERT for which females may be more vulnerable than males. ^{1}H-MRS appears to be a less sensitive technique for studying ecstasy's neurotoxic potential. 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.

Keywords

ecstasy, MDMA, neuroimaging, serotonin, SPECT, ^{1}H-MRSpectroscopy

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' (Gamma *et al*., 2005). 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 5-HT 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

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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 5-HT, concentrations in MDMA users and found either normal (Peroutka, 1987) or decreased levels (McCann *et al*., 1994; Ricaurte *et al*., 1990). Neuroendocrine challenge tests are another strategy for detecting 5-HT dysfunction by indirect means (Verkes *et al*., 2001). 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 MDMA on the living human brain. These imaging-techniques have identified a range of structural and functional consequences of MDMA 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' (*Time*, 2000) and 'Ecstasy Not Dangerous, Say Scientists' (*Guardian*, 2002). 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 (Ricaurte *et al*., 2002; retraction 2003). 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 (*Nature* editorial, 2004). MDMA supporters claim that studies showing cognitive deficiencies following MDMA use are methodologically flawed, and that there is no proof that a few doses of the drug will cause harm (*Nature* editorial, 2004). It is therefore all the more important to summarize what we do know and what we don't 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 focussing 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 (5-HT) 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 5-HT transporter densities (SERT), a known marker for 5-HT 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, 5-HT neurotoxicity is evidenced by losses in various markers unique to 5-HT neurones, such as 5-HT, 5-hydroxyindolacetic acid (5-HIAA), tryptophan hydroxylase, and 5-HT transporters (SERT) (Battaglia *et al*., 1987; Commins *et al*., 1987; Schmidt and Taylor, 1987; O'Hearn *et al*., 1988; Ricaurte *et al*., 1988a; Ricaurte *et al*., 1988b; Slikker *et al*., 1988; Insel *et al*., 1989; Molliver *et al*., 1990). Furthermore, it has been shown that MDMA-induced loss of 5-HT axonal markers is related to distal axonotomy (for review see Ricaurte *et al.* 2000). These studies further show that the effects of MDMA are selective, damaging brain 5-HT neurones. The effects of MDMA on 5-HT neurones 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 (Hatzidimitriou *et al*., 1999). 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 5-HT and 5-HIAA in rat brain persisting for 1 week or longer (O'Shea *et al*., 1998). 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 5-HT in monkey brain (Ricaurte *et al*., 1988a). 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 (McCann and Ricaurte, 2001), 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 (Mechan *et al*., 2006 in press). 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 5-HT, 5-HIAA, DA, or 3,4-dihydroxyphenylacetic acid (DOPAC) is therefore of particular interest (Fantegrossi *et al*., 2004).

Finally, brain levels of dopamine and its metabolite are not reduced by lower doses of MDMA, but are depleted after higher doses (Commins *et al*., 1987), suggesting that while MDMA is more toxic to 5-HT than to dopaminergic systems, it can also damage dopamine neurones.

In summary, MDMA administration at doses that approach those used recreationally by humans have consistently shown to cause selective injury to the 5-HT-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 5-HT 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 (^{1}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 5-HT 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 5-HT neurotoxicity (Scheffel *et al*., 1998; Reneman *et al*., 2002b; Szabo *et al*., 2002; de Win *et al*., 2004). 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 MDMA-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 5-HT neuron, and has been shown to be a reliable marker of the integrity of the 5-HT neuron (Zhou *et al*., 1998; Scheffel and Ricaurte, 1990). 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 5-HT 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 ^{11}C or ^{18}F), whereas SPECT utilizes radioligands with a longer half-life (such as ^{123}I and ^{99m}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 [^{11}C]McN5652 and [^{11}C]DASB; for SPECT [^{123}I]β-CIT.

PET studies Trans-1,2,3,5,6,10-β-hexahydro-6-[4-(methylthio) phenyl] pyrrolo-[2,1-∀] isoquinoline ([^{11}C]McN5652) is the first PET radioligand successfully developed in 1992 to label SERT in the living human brain (Boja *et al*., 1992). *In vitro*, the active enantiomer (+)-[^{11}C]McN5652 is a selective and potent inhibitor of 5-HT uptake. The *in vivo* regional distribution of (+)-[^{11}C]McN5652 in rats, baboons, and humans correlated with known regional concentrations of SERT, and the specific uptake of (+)-[^{11}C]McN5652 is blocked after pre-treatment with the 5-HT uptake blocker fluoxetine. In contrast, the brain uptake of the inactive enantiomer (−)-[^{11}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 [^{11}C]McN5652 were administered, was carried out in human ecstasy users (McCann *et al*., 1998). The purpose of the study was to compare [^{11}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 5-HT axonal markers, such as SERT, after treatment with ecstasy (Scheffel *et al*., 1998), this was the first report providing direct evidence that ecstasy users are susceptible to MDMA-induced brain 5-HT 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 ((+)-[^{11}C]McN5652 and (−)-[^{11}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* (Kuikka and Ahonen, 1999).

Buchert and others (2004) on the other hand used the cerebellum as a reference region for non-specific binding in a large PET study. 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 abstention 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-naive, and poly-drug control subjects, suggesting that the effects of ecstasy on SERT are reversible. However, subjects were scanned after a very short abstention period of minimal 3 days. Because both MDMA and [^{11}C]McN5652 bind to the SERT, one has to ensure that the SERT is available for [^{11}C]McN5652 binding. Although the time point after MDMA administration at which the SERT is free to bind is unknown, previous studies in humans have applied abstention periods of at least 3 weeks (McCann *et al*., 1998; Reneman *et al*., 2001). Because plasma levels of MDMA decline following a monoexponential model with a mean elimination half-life of about 8 hours (Mas *et al*., 1999) 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 [^{11}C]McN5652. Finally, exclusion of all subjects with an abstention 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 MDMA-induced neurotoxicity and not acute pharmacological effects of MDMA (Buchert *et al*., 2004).

Recently, McCann and others (2005) replicated their [^{11}C]McN5652 PET study in ecstasy users, using the cerebellum to control for non-specific binding. 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 [^{11}C]McN5652 DVs and duration of abstinence, suggesting that SERT may recover over time. However, unlike their previous study and unlike the Buchert study (2004), no reductions in SERT densities were observed in the midbrain and putamen. 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 (Hadzidimitriou *et al*., 1999). 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 reduced when assessed using [^{123}I]β-CIT (Reneman *et al*., 2002b). 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 (2005) study. However, it is well known that the 5-HT 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 (Table1). 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}C]McN5652, McCann *et al*. (2005) studied a new promising PET ligand for labeling SERT, [^{11}C]DASB ([11]C-amino-4-(2-dimethylaminomethylphenylsulfanyl)benzonitrile), which was tested in ecstasy-using subjects and controls. There was a high correlation between the two tracers, and no significant differences between the tracers were noted. Using [^{11}C]DASB, similar results to [^{11}C]McN5652 were obtained. The authors had hoped that because [^{11}C]DASB has a greater specific-to-non-specific equilibrium activity ratio than [^{11}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}C]DASB over [^{11}C]McN5652 is mainly related to a shorter scanning time (Huang *et al*., 2002). In addition, pretreatment with paroxetine displaced both ligands primarily from regions with high SERT densities (Szabo *et al*., 2002). Without correcting for multiple comparisons, no significant reductions in binding of [^{11}C]McN5652 or [^{11}C]DASB were observed in cortical regions (Szabo *et al*., 2002), suggesting that neither [^{11}C]McN5652 nor [^{11}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 (Parsey *et al*., 2000). This is a problem also encountered with [^{123}I]β-CIT SPECT, as will be discussed below.

SPECT studies The cocaine analogue 2β-carbomethoxy-3 β-(4-iodophenyl)tropane β-CIT is presently the best studied SPECT tracer for labelling of SERT. [^{125}]β-CIT binds with high affinity to both dopamine transporters (DAT) and SERT (Boja *et al*., 1991). The *in vivo* regional distribution of [^{123}I]β-CIT in rats, monkeys, and humans correlates well with known regional concentrations of SERT. The specific uptake of [^{123}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 5-HT reuptake inhibitors (Laruelle *et al*., 1993). By contrast, uptake of β-CIT in 5-HT-rich brain regions such as the brainstem, thalamus, and cerebral cortex can be blocked by 5-HT reuptake inhibitors (Laruelle *et al*., 1993; Farde *et al*., 1994; Kuikka *et al*.,

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

	Sample size *n*	Exposure (pills)	Overall SERT	SERT occipital cortex	SERT in midbrain	Moderate use	Gender	Reversibility	Correlation dose
PET									
McCann *et al.*, 1998*	29	880	↓ (SC + C)	Estimated −85% in males and females	Estimated −30%	NA	–	–	Lifetime dose
McCann *et al.*, 2005*	42	173	↓ (SC + C)	−54 & −68% in males and females	NS	NA	?	+	Typical dose
Buchert *et al.*, 2004	117	831	↓ (SC + C) in females; in males only in occipital and temporal cortex	>> −10%	Estimated −30%	NA	+	+	Typical dose
SPECT									
Semple *et al.*, 1999	20	672	↓ (C only)	−8% in males	NS in males	NA	Males only	+	Lifetime dose
Reneman *et al.*, 2001	69	530	↓ (SC + C) in females, not in males	NS in males, −16% in females	NS in males, −13% in females	–	+	+	Lifetime dose

* Estimated values from graphs, SC: subcortical brain areas, C: cortical brain areas, ?: unknown.

1995). 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 [^{123}I]β-CIT.

Ex vivo and *in vitro* studies in animals have shown that [^{123}I]β-CIT adequately detects changes in cortical as well as subcortical SERT densities secondary to 5-HT neurotoxicity, although cortical measurements must be interpreted with caution (Scheffel *et al*., 1992; Lew *et al*., 1996; Reneman *et al*., 2002b; de Win *et al*., 2004).

Several studies have been conducted using [^{123}I]β-CIT SPECT to study the effects of ecstasy on human brain 5-HT neurones. Semple and colleagues (1999) observed decreased [^{123}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. 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 (Heinz and Jones, 2000). Subjects were asked to abstain from psychoactive drugs for 1 week, and were scanned on average after an abstention period of 2.6 weeks. One cannot totally exclude that the results are at least in part influenced by acute pharmacological effects of MDMA, as discussed above for the Buchert study (2004). Furthermore, [^{123}I]β-CIT SPECT scans were acquired 90 minutes post-injection of the radiotracer. However, [^{123}I]β-CIT does not reach near-equilibrium conditions earlier than about 4 hours post-injection (Pirker *et al*., 2000). 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 [^{123}I]β-CIT binding to SERTs.

Using [^{123}I]β-CIT SPECT, we (Reneman *et al*., 2001) studied the effects of ecstasy in three different subgroups of 54 ecstasy users and 15 controls. 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 MDMA. 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 MDMA'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 (McCann *et al*., 1994). Furthermore, Liechti and co-workers reported more pronounced subjective responses to ecstasy in females than in males. These observations support the findings by us and Buchert (2004) that females may be more susceptible than males to the (neurotoxic) effects of ecstasy. 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 etiology of these gender differences is unknown, but may be related to differences in innate hormonal profiles (Kawas *et al*., 1997), volume and morphology of certain brain structures (Swaab *et al*., 1985), 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 (Lesch *et al*., 1996; discussed later).

Whether SPECT imaging with [^{123}I]β-CIT is sensitive enough to measure the density of serotonin transporters in areas of the cerebral cortex is controversial, and subject to debate (Heinz and Jones, 2000; Ricaurte and McCann, 2001). 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 (Farde *et al*., 1994) 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}I]β-CIT in assessing SERT densities (de Win *et al*., 2005). In a double-blind, placebo-controlled, cross-over design the effect of the selective SSRI citalopram on [^{123}I]β-CIT binding was assessed in cortical as well as subcortical brain regions. After citalopram treatment [^{123}I]β-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}I]β-CIT SPECT is a valid technique in studying SERT densities in 5-HT-rich brain regions such as the midbrain and (hypo)thalamus. However, even though [^{123}I]β-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}C]DASB and [^{11}C]McN5652 PET.

Another problem associated with [^{123}I]β-CIT is its affinity for both 5-HT and DAT. The midbrain, thalamus, and cortex also contain DAT besides SERT. However, displacement studies in animals (Scheffel *et al*., 1992; Farde *et al*., 1994) have shown that binding of β-CIT is predominantly associated to SERT in these brain regions. Furthermore, since we (Reneman *et al*., 2002c) and Semple *et al.* (1999) did not observe reductions in striatal [^{123}I]β-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

^{1}H-MRS studies Magnetic resonance spectroscopy (MRS) 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 *N*-acetylaspartate (NAA) detected by proton magnetic resonance spectroscopy (^{1}H-MRS) represents a robust but non-specific marker for neuronal loss or dysfunction (Urenjak *et al*., 1993). Although the use of NAA has not been previously validated for detecting MDMA-induced neurotoxicity, unlike SERT, PET, and SPECT, NAA has been shown to be predominantly localized to neurones, axons, and dendrites within the central nervous system (Simmons *et al*., 1991). Studies of diseases known to involve neuronal and/or axonal loss (infarcts, brain tumours, 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 (Guimaraes *et al*., 1995; Strauss *et al*., 1997). 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.

In 1999 Chang and colleagues reported the first findings on ^{1}H-MRS spectra obtained in 22 ecstasy users and 37 controls, who had to abstain from psychoactive drugs for at least 2 weeks. 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 (Reneman *et al*., 2002a), studied at least 1 week after the last ecstasy tablet taken, as compared to 12 gender and age matched control subjects. 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 ^{1}H-MRS, and may therefore also account for the discrepancy between the studies.

In an exploratory study, hippocampal ^{1}H-MRS spectra of five ecstasy users were compared with those of controls with no history of substance abuse (Obergriesser *et al*., 2001). No differences between users and controls were observed. Furthermore, Daumann and colleagues (2004) recently compared ^{1}H-MRS spectra of 13 ecstasy users with 13 controls. 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 all have found reductions in SERT density in heavy ecstasy users with the use of different techniques and different radioligands. In all but one study (Semple *et al*., 1999) 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 more vulnerable than males (Reneman *et al*., 2001; Buchert *et al*., 2004). In the third study that did not find gender differences (McCann *et al*., 1998), 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 5-HT axonal terminals in some but not all brain regions (Hatzidimitriou *et al*., 1999). With respect to the use of ^{1}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, ^{1}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 [^{123}I]β-CIT SPECT by Semple *et al.* (1999) and Reneman (2001) (in the order of 10%) are similar. However, this is not the case when compared with McCann (1998, 2005; estimated from graph at −54% to −85%; Table 1) using [^{11}C]McN5652 and [^{11}C]DASB PET. Previous PET studies in animals have shown that PET and SPECT underestimate the true extent of SERT loss by approximately 40–50% (Szabo *et al*., 2002; de Win *et al*., 2004). In MDMA-treated baboons, reductions in the order of 30% in most regions were observed with a maximum of 42% in the occipital cortex (Szabo *et al*., 2002). 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 5-HT (Kish *et al*., 2005). This will result in an overestimation of SERT densities, and consequently an underestimation of ecstasy's neurotoxic effects (Kish *et al*., 2005). 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 (2004) and McCann study (2005) 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 move-

ment 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 5-HT may enable the measurement of acute fluctuations of 5-HT, such as stress. A ligand insensitive to competition provides a more reliable measure of SERT unaffected by levels of 5-HT. This seems to be particularly true for [123I]β-CIT SPECT, and not as much for [11C]DASB (and possibly [11C]McN5652) PET), as β-CIT can be displaced by endogenous 5-HT (Heinz *et al*., 2004), whereas [11C]DASB binding to SERT declined after acute reduction of 5-HT levels (Milak *et al*., 2005). 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 5-HT concentrations (Ramamoorthy and Blakely, 1999). Acutely, 5-HT will prevent sequestration of SERT (Ramamoorthy and Blakely, 1999). However, if 5-HT concentrations are low for a long period of time, homeostatic mechanisms may reduce synthesis of the SERT protein as a 'neuroadaptive' response to low 5-HT levels, in which low levels of 5-HT will lead to increased sequestration and reduced synthesis of the SERT (Linnet *et al*., 1995). Although a variety of animal studies demonstrated that reductions in immunostained 5-HT axons and axon terminals are associated with decreases in 5-HT 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 5-HT 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 5-HT and 5-HIAA levels but not SERT, whereas the 5-HT neurotoxin p-chloroamphetamine reduced not only 5-HT and 5-HIAA but SERT as well (Dewar et al., 1992). This study indicates that SERT reductions after p-chloroamphetamine administration are not a consequence of prolonged 5-HT depletions, but reflect p-chloroamphetamine induced brain 5-HT 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 et al., 2002 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 (Szabo *et al*., 2002). Although SERT measurements with PET and SPECT in the neocortex should be interpreted with caution, the large preclinical literature demonstrating toxicity of MDMA toward 5-HT 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 (Curran, 2000). 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 (de Win *et al*., 2005).

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 5-HT-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_2 receptor densities, FDG PET, functional MRI (*f*MRI), perfusion and diffusion MRI, and cognitive studies are all indicative of alterations of brain (5-HT) 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) ([^{123}I]ADAM), which has high binding affinity and selectivity toward SERT (Choi *et al*., 2000; Newberg *et al*., 2005). 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 *f*MRI, 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 MDMA may be neurotoxic in human beings (Gijsman *et al*., 1999; McCann and Ricaurte 2001). Ecstasy users may be studied prospectively to shed light on the fate of damaged 5-HT neurones 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 focussing on other systems than the serotonergic system to increase our understanding on the effects of MDMA and subsequent compensatory mechanisms in the brain. Because SERT plays a key element in the regulation of synaptic 5-HT 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 (Roiser *et al*., 2005), although we did not observe such an effect (Reneman *et al*., in press). 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 5-HT 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 5-HT depletion and long-term anxiety (Morley *et al*., 2004) has been shown, whereas the combination of MDMA with ethanol may result in long-term consequences on pre-synaptic modulation of hippocampal 5-HT release (Cassel *et al*., 2005). 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 5-HT 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 ^{1}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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