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Investigating the potential neurotoxicity of Ecstasy (MDMA): an imaging approach

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Human users of 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') users may be at risk of developing MDMA-induced neuronal injury. Previously, no methods were available for directly evaluating the neurotoxic effects of MDMA in the living human brain. However, development of in vivo neuroimaging tools has begun to provide insights into the effects of MDMA in the human brain. In this review, contributions of brain imaging studies on the potential neurotoxic effects of MDMA and functional consequences are highlighted. An overview is given of PET, SPECT and MR spectroscopy studies, most of which show evidence of neuronal injury in MDMA users. Different neuroimaging tools are discussed that have investigated potential functional consequences of MDMA-induced 5-HT neurotoxic lesions. Finally, the contribution of brain imaging in future studies is discussed, emphasising the crucial role it will play in our understanding of MDMA's short- and long-term effects in the human brain. Copyright © 2001 John Wiley & Sons, Ltd.

KEY WORDS — MDMA; neurotoxicity; PET; SPECT; MRI; review

INTRODUCTION

Findings in animals suggest that the popular recreational drug 3,4-methylenedioxymethamphetamine (MDMA or Ecstasy) might damage brain serotonin (5-HT) neurones in human beings. MDMA-induced 5-HT neurotoxicity has been demonstrated in animals, including primates, using a variety of experimental techniques at doses that approach or overlap those used recreationally by human beings. In these animals, 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 (Battaglia *et al.*, 1987; Commins *et al.*, 1987; Insel *et al.*, 1989; Molliver *et al.*, 1990; O'Hearn *et al.*, 1988; Ricaurte *et al.*, 1988a; Ricaurte *et al.*, 1988b; Schmidt, 1987; Slikker *et al.*, 1988). The effects of MDMA are highly selective, exclusively 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, others remain denervated up to 7 years after treatment with MDMA (Hatzidimitriou *et al.*, 1999).

If MDMA does produce 5-HT neurotoxicity in humans, there would be important ramifications for the mental health and psychological function of people who use this drug, because irreversible loss of 5-HT neurones may be responsible for an immediate or delayed onset of neuropsychiatric disorders in which 5-HT has been implicated. Specifically, 5-HT imbalance has been postulated to underlie psychiatric disorders including depression, anxiety, panic disorder and disorders of impulse control. In line with this, there have been many case reports of neuropsychiatric sequelae after MDMA use,

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including paranoid psychosis, anxiety, depression and panic disorder (Hegadoren *et al.*,1999; Schifano *et al.*, 1998). Furthermore, since 5-HT appears to play an important role in cognitive function, and the greatest neurotoxic effects of MDMA in animals are observed in the frontal cortex and hippocampus (areas known to play crucial roles in cognitive function and memory), it is also important to study these effects of MDMA in the human brain.

Previously, potential 5-HT neurotoxic changes in the living human brain have only been identified using indirect methods. For example, some studies have evaluated the cerebrospinal fluid 5-HIAA 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, 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. Furthermore, these imaging techniques have been shown to identify several potential functional consequences of MDMA-induced neurotoxicity and may be useful in identifying unknown but potential long-term effects.

The purpose of this review is to present 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, and how such imaging tools could be used in future studies. Table1 summarises the main studies that have investigated the effects of MDMA in humans using neuroimaging techniques.

BIOLOGICAL MARKERS OF NEURONAL INJURY

Studying 5-HT neuronal loss using PET and SPECT

The introduction of an increasing number of radioactive tracers and the development of special detecting systems have enabled the detection of molecules in vivo and the production of functional images of brain chemistry. The most advanced detection systems nowadays are PET and SPECT. PET uses relatively short-lived positron-emitting isotopes (such as ^{11}C or ^{18}F), whereas SPECT utilises radioligands with a longer half-life (such as ^{123}I and ^{99m}Tc). Spatial resolution of the 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 logistics and production of SPECT radiotracers, this technique is more widely available than PET.

The 5-HT transporter is a structural element of the 5-HT neuron and is thought to be a reliable marker of the integrity of the 5-HT neuron (Zhou *et al.*, 1998; Scheffel and Ricaurte, 1990). Recently, PET and SPECT radioligands have been developed for neuroimaging of 5-HT transporters in the human brain. Because animal studies already showed that MDMA-induced neurotoxicity is associated with loss of 5-HT neurones, imaging of the 5-HT transporter with PET or SPECT is ideally suited for studying the potential neurotoxic effects of MDMA in the living human brain. However, because there are important features for a good in vivo tracer for 5-HT transporters, only a few radioligands fulfil at least some of the criteria, and subsequently only two tracers—[^{11}C] McN5652 and [^{123}I] β—CIT–have been used to investigate the effects of MDMA in vivo.

Table 1. Summary of neuroimaging studies investigating effects of MDMA in the human brain

Investigation	Biological marker	Technique used	Reference
Neuronal loss	5-HT transporter	[^{11}C]McN5652 PET	McCann *et al.*, 1998
		[^{123}I]β-CIT SPECT	Semple *et al.*, 1999; Reneman *et al.*, 2001a, 2001c
	NAA	^{1}H-MRS	Chang *et al.*, 1999
	ADC	Diffusion MRI	Reneman *et al.*, 2001d
Functional consequences	5-HT$_2$ receptor	[^{123}I]R91150 SPECT	Reneman *et al.*, 2000b, 2001b
	Cerebral blood flow	[^{99m}Tc] HMPAO SPECT	Chang *et al.*, 2000
		Perfusion MRI	Reneman *et al.*, 2000a, 2001e
	Cerebral glucose metabolic rate	FDG PET	Obrocki *et al.*, 1999; Buchert *et al.*, 2001
	Cognitive function	FMRI	Gamma *et al.*, 2001
Linking neuronal injury with cognitive function	5-HT$_2$ receptor + memory	[^{123}I]R91150 SPECT	Reneman *et al.*, 2000b
	5-HT transporter + memory	[^{123}I]β-CIT SPECT	Reneman *et al.*, 2001c
	NAA + memory	^{1}H-MRS	Reneman *et al.*, 2001d

Trans-1,2,3,5,6,10-β-hexahydro-6-[4-(methylthio)-phenyl] pyrrolo-[2,1-$\forall$] isoquinoline ([^{11}C]McN5652) was the first PET radioligand (successfully developed in 1992) to label 5-HT transporters 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 5-HT transporters, and the specific uptake of (+)-[^{11}C]McN5652 is blocked after pretreatment 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. Scheffel and co-workers (1998) were the first to validate the use of [^{11}C]McN5652 PET in detecting MDMA-induced 5-HT neuronal loss. To this purpose, following baseline scans with [^{11}C]McN5652 PET, a baboon was treated with MDMA. PET studies at 13, 19 and 40 days post-MDMA treatment revealed decreases in mean radioactivity of (+)-[^{11}C] McN5652, but not (−)[^{11}C]McN5652, in all brain regions studied. Reductions in specific [^{11}C] McN5652 binding (calculated as the difference in radioactivity concentrations between (+) and (−)[^{11}C]McN5652) ranged from 44% in the pons to 89% in the occipital cortex. Data obtained from PET studies correlated well with regional 5-HT axonal marker concentrations in the CNS measured after sacrifice of the animal, although [^{11}C]McN5652 PET tended to underestimate the extent of 5-HT damage found postmortem. Therefore it was concluded that, using [^{11}C]McN5652 PET, it should be possible to determine whether human MDMA users are susceptible to MDMA's neurotoxic effects.

Subsequently, after having validated [^{11}C] McN5652 PET for detecting MDMA-induced 5-HT neuronal loss, a PET study with [^{11}C]McN5652 was carried out in 1998 in human MDMA users (McCann *et al.*, 1998). The purpose of the study was to compare [^{11}C]McN5652-labelled 5-HT transporter densities in human MDMA users with 5-HT transporter densities in control subjects. Nine males and four females who reported previous use of MDMA 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. As in the MDMA-treated baboon, global decreases in 5-HT transporter densities were observed in the MDMA users, which correlated with the extent of previous MDMA use. Taken in conjunction with the results of previous animal studies that showed selective decreases in 5-HT axonal markers after treatment with MDMA (Scheffel *et al.*, 1998), this was the first report providing direct evidence that MDMA users are susceptible to MDMA-induced brain 5-HT neuronal injury.

Since then, similar observations have been made using [^{123}I]β-CIT SPECT. The cocaine analogue 2β-carbomethoxy-3 β-(4-iodophenyl)tropane (β-CIT) is presently the best studied and most used SPECT tracer for labelling of 5-HT transporters. [^{11}C]β-CIT binds with high affinity to both dopamine and 5-HT transporters (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 5-HT transporters. The specific uptake of [^{123}I]β-CIT in the striatum is primarily associated with dopamine agonist (DA) transporters, since it is blocked by the selective DA uptake inhibitor GBR 12909 but not by selective 5-HT uptake 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 uptake inhibitors (Farde *et al.*, 1994; Kuikka *et al.*, 1995; Laruelle *et al.*, 1993). Thus, these studies indicate that in selected brain areas, e.g. brainstem, thalamus, cerebral cortex and other regions in which 5-HT transporter densities far exceed those of DA transporters, it is possible to estimate 5-HT transporter 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 5-HT transporter densities secondary to 5-HT neurotoxicity (Lew *et al.*, 1996; Scheffel *et al.*, 1992a). To validate the use of [^{123}I]β-CIT in combination with SPECT in detecting 5-HT neurotoxic lesions, recently a study was undertaken in a rhesus monkey (submitted for publication). Following baseline [^{123}I]β-CIT SPECT scans, the monkey was treated with MDMA. SPECT studies at 4, 10 and 31 days post-MDMA treatment revealed decreases in [^{123}I]β-CIT binding ratios in the hypothalamic/midbrain region. Data obtained from SPECT studies in this brain region correlated well with regional 5-HT transporter densities obtained with autoradiography after sacrifice of the animal.

One other study has investigated SPECT measurement of reductions in 5-HT transporter densities after MDMA treatment. In that study the 5-HT transporter ligand 5-iodo-6-nitroquipazine (INQUIP) was used in control and MDMA-treated rhesus monkeys (Jagust *et al.*, 1996). [^{123}I]INQUIP was able to detect some cortical as well as subcortical reductions in 5-HT transporters in the MDMA-treated monkeys when compared with the non-treated monkeys.

Several recent studies have used [^{123}I]β-CIT SPECT to study the effects of MDMA 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 10 male MDMA users, compared with 10 well-matched controls. Reduction in binding was inversely correlated with the time since last MDMA use. No correlations were observed between [^{123}I]β-CIT binding ratios and a variety of neuropsychological measures. However, several problems are associated with this study. Subjects were asked to abstain from psychoactive drugs for 1 week, whereas a period of 3 weeks was used in the [^{11}C]McN5652 PET study (McCann *et al.*, 1998). Furthermore, [^{123}I]β-CIT SPECT scans were acquired 90 min after injection of the radiotracer. However, [^{123}I]β-CIT does not reach near-equilibrium conditions earlier than about 4 h after injection (Pirker *et al.*, 2000). At scanning times this early, factors related to radioligand delivery and washout, rather than 5-HT transporter binding per se, play a prominent role in determining [^{123}I]β-CIT binding.

Using [^{123}I]β-CIT SPECT, Reneman and colleagues (2001a) replicated findings of previous PET and SPECT studies suggesting that heavy use of MDMA is associated with neurotoxic effects on 5-HT neurones in several 5-HT-rich brain regions. Three different subgroups of 54 MDMA users and 15 controls were scanned after a drug-free interval of at least 3 weeks. Subjects were recruited from the same community sources and thus were well matched for age, gender distribution and psychosocial factors. Interestingly, the authors observed significant decreases in overall binding ratios in female but not in male heavy MDMA users, suggesting that females may be more susceptible than males to the neurotoxic effects of MDMA. It was also observed that MDMA-induced neurotoxic changes in most, but not all, brain regions of female ex-MDMA users were reversible, and that moderate MDMA use may lead to neurotoxic changes in the parieto-occipital cortex and occipital cortex, brain regions which seem to be particularly sensitive to MDMA's effects.

Regional differences in 5-HT transporter densities reported in MDMA users studied with [^{123}I]β-CIT SPECT by Semple *et al.* and Reneman *et al.* are fewer than in the study of McCann and colleagues. This may reflect higher non-specific binding of [^{123}I]β-CIT. However, data obtained from [^{123}I]β-CIT SPECT studies in the MDMA-treated rhesus monkey correlated well with regional 5-HT transporter densities obtained with autoradiography in some brain regions. Like [^{11}C]McN5652 PET, [^{123}I]β-CIT SPECT may also lack adequate sensitivity to detect smaller MDMA-induced 5-HT lesions.

Studying non-specific neuronal loss using ^{1}H-MR spectroscopy

The reduction of the amino acid *N*-acetylaspartate (NAA) detected by proton magnetic resonance spectroscopy (^{1}H-MRS) is a robust but unspecific marker for neuronal loss or dysfunction (Urenjak *et al.*, 1993). In addition to NAA, myo-inositol (MI, a possible glial marker) and creatine/phosphocreatine (Cr) can be assessed. Determining NAA changes in relation to Cr is commonly employed and seems valid, because Cr remains stable in a variety of brain diseases. In 1999 Chang and colleagues reported findings on ^{1}H-MRS spectra obtained in 22 MDMA users and 37 controls, who had to abstain from psychoactive drugs for at least 2 weeks. Normal NAA levels were observed in MDMA users, but MI and MI/Cr levels were increased in the parietal white matter of MDMA users. The cumulative lifetime MDMA dose showed significant effects of MI in the parietal white matter and the occipital cortex. The normal NAA levels suggest a lack of significant neuronal injury in MDMA users, whereas increased MI may reflect increased glial content, possibly reflecting ongoing repair processes.

In contrast, Reneman and co-workers (submitted for publication) recently observed decreased NAA/Cr and NAA/Cho levels in the frontal cortex of 15 male MDMA users, studied at least 1 week after the last MDMA tablet taken, compared with 12 control subjects matched for gender and age. Furthermore, a significant association was observed between extent of previous MDMA use and NAA/Cr or NAA/Cho ratios in the frontal cortex. Discrepancies between the study by Chang *et al.* and that of Reneman *et al.* may be attributed in part to age-associated differences between the studies. In the Reneman study, subjects (both MDMA 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 discrepancies between the studies.

FUNCTIONAL CONSEQUENCES OF MDMA-INDUCED NEURONAL INJURY

5-HT is involved in various brain functions, such as mood, sleep, appetite and cognitive function. In addition, considerable evidence has accumulated

suggesting that 5-HT plays a pivotal role in the regulation of postsynaptic 5-HT_2 receptor densities (for review see Meneses, 1999) and control of cerebral perfusion (Belohlavkova *et al.*, 2001; Cohen *et al.*, 1996). While a number of studies have, using neuropsychological assessments, shown evidence for cognitive problems in MDMA users (for review see Parrott, 2000), few neuroimaging studies have simultaneously investigated potential functional consequences of MDMA-induced 5-HT neurotoxic lesions. This lack of studies is probably related to the fact that these functions are difficult to study using functional neuroimaging techniques, except for techniques focusing on cerebral perfusion and cerebral glucose metabolic rate, i.e. techniques indicating general neuronal metabolic activity. Although some authors have investigated the acute effects of Ecstasy on glucose metabolism and cerebral blood flow (Schreckenberger *et al.*, 1998, 1999; Gamma *et al.*, 2000), this review concentrates on the long-term effects of MDMA-induced 5-HT neurotoxic lesions.

Postsynaptic 5-HT_2 receptor densities

While [^{11}C]McN5652 and [^{123}I]β-CIT SPECT study presynaptic 5-HT transporter densities, the development of iodine-123-4-amino-N-[1-[3[(4-fluorophenoxy)propyl]4-methyl-4-piperidinyl]5-iodo-2-methoxybenzamide ([^{123}I]R91150), a radioligand which binds with high affinity and selectivity to 5-HT_{2A} receptors (Terriere *et al.*, 1995), has made it possible to assess the density of cortical HT_{2A} receptors in the living human brain using SPECT (Busatto *et al.*, 1997). While the effects of MDMA on 5-HT nerve fibres and terminals have been studied extensively in animals, little is known about its effects on postsynaptic 5-HT receptors. Only one study has evaluated postsynaptic 5-HT_2 receptor densities in MDMA-treated rats (Scheffel *et al.*, 1992b). There is considerable evidence from the literature that postsynaptic 5-HT_2 receptors manifest a down-regulation in situations with high levels of synaptic 5-HT, while 5-HT depletion has been associated with a compensatory up-regulation of 5-HT_2 receptors (Sharif *et al.*, 1989; Stockmeier and Kellar, 1989). Therefore, Reneman and colleagues (2001b) studied cortical 5-HT_{2A} receptor densities in the cerebral cortex of 17 recent and seven ex-MDMA users using [^{123}I]R91150 SPECT. Cut-off points of the drug-free interval of 2 months in the ex-MDMA group and 1 week in the recent MDMA group were chosen. The authors report that postsynaptic 5-HT_{2A} receptor densities were significantly lower in all cortical areas studied, while 5-HT_{2A} receptor densities were significantly higher in the occipital cortex of ex-MDMA users. This result suggests a compensatory up-regulation of postsynaptic 5-HT_{2A} receptors in the occipital cortex of ex-MDMA users, possibly because of low synaptic 5-HT levels.

Cerebral blood flow

SPECT and perfusion MR imaging have both been employed to study the effects of MDMA-induced 5-HT alterations on brain cerebrovasculature of MDMA users. Chang and colleagues (2000) compared regional cerebral blood flow (rCBF) in 21 MDMA users and 21 age- and gender-matched control subjects using [^{99m}Tc] hexamethylpropyleneamine oxime (HMPAO) SPECT. In addition, 10 of the MDMA subjects were scanned after receiving two doses of MDMA. Eight subjects were scanned within 3 weeks after they received MDMA, while two were scanned more than 2 months later. The 21 MDMA users showed no different regional or global rCBF compared with controls. However, 3 weeks after MDMA administration, rCBF was decreased, implicating vasoconstriction, in several cortical brain regions and the caudate nucleus, whereas rCBF tended to be increased, implicating vasodilatation, rather than decreased in the two subjects who were scanned 2–3 months after MDMA administration. The short-term effect of MDMA involves excessive release, and it was therefore suggested that with normalisation of the excess of 5-HT or depletion of 5-HT in some regions at a later time point, rCBF may return to normal or increase above normal, due to removal of serotonergic constrictive effects.

Similar observations were made by Reneman and co-workers (2000a). In order to examine whether changes in brain 5-HT_{2A} receptor densities are associated with alterations in blood vessel volumes, [^{123}I]R91150 SPECT and perfusion-weighted MRI was performed in five MDMA users and six control subjects. MDMA-using subjects were scanned after a drug-free interval of 7 weeks on average. Using dynamic contrast-enhanced perfusion-weighted MR imaging, it has become possible to study the brain vasculature by calculating rCBV maps (Rosen *et al.*, 1989). In specific brain regions, high cortical 5-HT_2 receptor densities, suggestive of low synaptic 5-HT levels, were correlated with high rCBV values, implicating vasodilatation, whereas low cortical 5-HT_2 receptor densities, suggestive of high synaptic 5-HT levels, were correlated with low rCBV values, implicating vasoconstriction. These findings suggest that

MDMA may affect brain cerebrovasculature. Future studies in animals would be most useful in clarifying specific effects of MDMA on cerebral blood flow and vasculature.

Cerebral glucose metabolic rate

Two studies used 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG) PET as an index of glucose metabolism. In the first, PET scans were performed 2–16 months after the last MDMA ingestion in seven MDMA users and seven age-matched tumour patients (Obrocki *et al.*, 1999). It was postulated that MDMA-induced 5-HT neurotoxic lesions may lead to alterations in glucose utilisation. Glucose metabolic uptake of the MDMA-using group was reduced in the left hippocampus, a brain region known to be consistently affected by MDMA in animals treated with this drug. Recently, a second study by the same group was published in which FDG PET scans were performed between 3 days and 96 months after the last MDMA ingestion in 93 MDMA users and 27 age-matched oncology patients (Buchert *et al.*, 2001). FDG uptake was reduced in the putamen, caudate and left amygdala. No association between FDG uptake and extent of previous MDMA use was observed. Because differences between MDMA users were rather small and restricted to some brain regions, the authors concluded that FDG PET cannot be used as a diagnostic tool in detecting MDMA-induced neuronal loss, and should rather be used complementary with other imaging tools such as PET and SPECT.

LINKING BIOLOGICAL MARKERS OF NEURONAL INJURY WITH IMPAIRED COGNITIVE FUNCTION

By combining imaging studies with neuropsychological assessment it is possible to study links between neuronal loss, or brain damage, and cognitive function. Several studies have found an association between markers of neuronal injury and impaired cognitive function in MDMA users. Reneman and colleagues (2000b) investigated whether MDMA use is related to compensatory alterations in postsynaptic 5-HT$_{2A}$ receptors and whether there is a relation between the latter and memory disturbances. Memory is of particular interest, as several studies have found that recreational MDMA users display significant memory impairments, whereas their performance on other cognitive tests is generally normal (Parrott, 2000). To this purpose brain cortical 5-HT$_{2A}$ receptor densities were studied with [^{123}I]-5-I-R91150 SPECT in five abstinent MDMA users and nine healthy controls. Memory performance was assessed using a word recall test, the Rey Auditory Verbal Learning Test (RAVLT). [^{123}I]-5-I-R91150 binding ratios were significantly higher in the occipital cortex of MDMA users than in controls, indicating up-regulation. Mean cortical 5-HT$_{2A}$ receptor binding correlated positively with RAVLT recall in MDMA users, suggesting altered 5-HT neuronal function with correlated memory impairment in abstinent MDMA users.

In another study, Reneman and colleagues (2001c) compared cortical [^{123}I]β-CIT labelled 5-HT transporter densities in different groups of MDMA users: 22 recent MDMA users who had not used MDMA for at least 3 weeks, 16 ex-MDMA users who had stopped using MDMA for more than 1 year, and 13 controls who claimed never to have used MDMA. In addition, memory was assessed using the RAVLT. Reduced cortical 5-HT transporter densities were observed in recent but not ex-MDMA users. However, both recent and ex-MDMA users recalled significantly fewer words than the controls. Greater use of MDMA was associated with greater impairment in immediate verbal memory. However, memory performance was not associated with [^{123}I]β-CIT binding to cortical 5-HT transporters, in contrast to the previously mentioned study by Reneman *et al.* (2000b), in which a strong association between postsynaptic 5-HT$_2$ receptor densities and memory performance was observed. These findings suggest that while the neurotoxic effects of MDMA on 5-HT neurones in the human cortex may be reversible, the effects of MDMA on memory function may last longer.

Finally, one other study has investigated the relation between brain damage and memory function (Reneman *et al.*, 2001d). Again, the RAVLT was used to study eight abstinent MDMA users and seven controls. In addition, ^{1}H-MRS was used in different brain regions of all MDMA users to measure NAA/Cr ratios. MDMA users recalled significantly fewer words than the controls. In MDMA users, delayed memory function was strongly associated with NAA/Cr only in the prefrontal cortex, suggesting that greater decrements in memory function predicted lower NAA/Cr levels—and by inference greater neuronal dysfunction—in the prefrontal cortex of MDMA users.

Although most of these studies had small sample sizes, they at least suggest an intriguing relationship between markers of brain damage and memory performance in MDMA users. However, their results need to be confirmed in studies with larger numbers of subjects.

OTHER NEUROIMAGING TECHNIQUES

Functional MR imaging (FMRI) may have tremendous potential for better delineating the consequences of MDMA-induced neurotoxicity. FMRI is a novel imaging technique aimed at localising cerebral functions, including sensorimotor, vision, language and memory functions (Belliveau *et al.*, 1990). The technique is based on the phenomenon that activation of specific brain areas causes changes in haemodynamics and oxygenation of the cerebral blood at these locations, which can be visualised with echo-planar MR imaging techniques. FMRI may be particularly useful in visualising brain activity patterns that correspond with cognitive functions in MDMA users, because several research groups have found MDMA users to have cognitive deficits, particularly on memory tasks. A study similar to FMRI was recently conducted by Gamma and colleagues (2001), in which rCBF profiles obtained with [$H_2{}^{15}O$] PET were compared in MDMA users and controls during cognitive activation by a task requiring sustained attention. No differences between the two groups were observed. Because the bulk of studies utilising classic neuropsychiatric testing have reported deficits in memory function (Bolla *et al.*, 1998), whereas performance on more basic cognitive tasks is generally unimpaired (Parrott, 2000), it would be of interest to compare rCBF profiles of MDMA users and controls during activation by a memory task. However, FMRI may prove to be a more valuable technique in identifying MDMA-induced functional consequences, as the technique is even faster than [$H_2{}^{15}O$] PET, without engendering more radiation dosimetry with each sampling and with fine temporal resolution.

Another technique of interest is diffusion-weighted MR imaging, which provides a unique form of MR contrast that enables the diffusional motion of water molecules to be quantitatively measured in biological tissue, especially axons (Le Bihan *et al.*, 1992). Cellular structures, such as highly organised myelinated axons in white matter, restrict water molecular motion, and the apparent diffusion coefficient (ADC) is less than in bulk water (Moseley *et al.*, 1990; Pierpaoli *et al.*, 1996). Any process that results in changes in structural elements of tissue, removing some of the 'restricting' barriers, can result in increased ADC. It is therefore thought that diffusion-weighted MR imaging is a promising approach for the evaluation of tissue changes in degenerating brain and nerve matter (Horsfield *et al.*, 1998; Kinoshita *et al.*, 1999). In a preliminary study Reneman and colleagues (2001e) observed increased ADCs in the globus pallidus of eight MDMA users, compared with six controls matched for age and gender. These changes in the globus pallidus may reflect (non-5-HT-specific) tissue change, which is in agreement with case reports suggesting that the globus pallidus is particularly sensitive to the effects of MDMA (Spatt *et al.*, 1997).

In the near future, diffusion tensor imaging (DTI) may turn out to be a useful technique in studying axonal projections in the living human brain. It enables in vivo three-dimensional reconstruction of axonal projections using a rapid 3D high-resolution diffusion-weighted imaging technique combined with a recently designed fibre reconstruction algorithm (Xue *et al.*, 1999). However, it is of great importance that animal studies are conducted in parallel to studies in humans to validate this technique, and others mentioned in the present report, in detecting MDMA-induced 5-HT neuronal lesions.

DISCUSSION

In summary, several lines of evidence from brain imaging techniques provide evidence that human MDMA users are susceptible to MDMA-induced neuronal damage, and that this may lead to functional impairments such as memory loss and, possibly, alterations in the brain cerebrovasculature.

It is important to note that the studies discussed here are limited by a number of factors. Their conclusions depend heavily on results of experimental animal studies showing MDMA-induced serotonergic lesions. Studies in humans are clearly subject to ethical and methodological constraints (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 causative links can be implied between MDMA use and neurotoxicity. Clearly, to definitively establish a causative link between observed neurotoxic changes and MDMA use, an experimental study design would be needed. However, given that the drug is illicit, has potential neurotoxicity and has resulted in some fatalities, it is very difficult to perform such a study. One possible approach would be to assess people both before and after they took MDMA. Neuroimaging techniques may be very helpful in providing such longitudinal studies in human MDMA users.

Whereas both PET and SPECT have become common techniques in assessing the potential risk of MDMA, more recent MR imaging tools hold great promise but have yet to prove their potential in the field. Currently available radioligands may not have

the requisite sensitivity to detect smaller MDMA-induced lesions. Without doubt more selective radioligands for the 5-HT transporter will be developed for PET or SPECT in the future that may be more sensitive in detecting MDMA-induced neuronal loss. Although none of the currently available techniques is perfect, converging lines of evidence are needed (using combinations of different imaging techniques) to make an adequate risk assessment of MDMA. In any case, the currently available preliminary data obtained using these methods can be strengthened considerably by laying the groundwork with preclinical studies in animals where direct, postmortem neurochemical and neuroanatomical studies can be conducted.

Future studies will have to determine whether neurotoxic effects in heavy MDMA users tested to date also occur in less frequent users. Some have argued that even a single dose of MDMA may be neurotoxic in humans (Gijsman *et al.*, 1999; McCann and Ricaurte, 2001). MDMA 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 memory loss. Finally, more studies should be conducted focusing on other systems than the serotonergic system to increase our understanding of the effects of MDMA and subsequent compensatory mechanisms in the brain.

If indeed MDMA leads to 5-HT neuronal injury, the health implications may be considerable, in which MDMA will be responsible for early or late neuropsychiatric morbidity. Neuroimaging techniques will greatly contribute to our understanding of MDMA'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 important feature.

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