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Designer drugs: how dangerous are they?

L. Reneman

Department of Radiology, Academic Medical Center, Amsterdam, The Netherlands

Summary. Of the designer drugs, the amphetamine analogues are the most popular and extensively studied, ecstasy (3,4-methylenedioxymethamphetamine; MDMA) in particular. They are used recreationally with increasing popularity despite animal studies showing neurotoxic effects to serotonin (5-HT) and/or dopamine (DA) neurones. However, few detailed assessments of risks of these drugs exist in humans. Previously, there were no methods available for directly evaluating the neurotoxic effects of amphetamine analogues in the living human brain. However, development of *in vivo* neuroimaging tools have begun to provide insights into the effects of MDMA in human brain. In this review, contributions of brain imaging studies on the potential 5-HT and/or DA neurotoxic effects of amphetamine analogues will be highlighted in order to delineate the risks these drugs engender in humans, focusing on MDMA. An overview will be given of PET, SPECT and MR Spectroscopy studies employed in human users of these drugs. Most of these studies provide suggestive evidence that MDMA is neurotoxic to 5-HT neurones, and (meth)amphetamine to DA neurones in humans. These effects seem to be dose-related, leading to functional impairments such as memory loss, and are reversible in several brain regions. However most studies have had a retrospective design, in which evidence is indirect and differs in the degree to which any causative links can be implied between drug use and neurotoxicity. Therefore, at this moment, it cannot be ascertained that humans are susceptible to MDMA-induced 5-HT injury or (meth)amphetamine-induced DA injury. Finally, although little is known about other amphetamine analogues there are important questions as to the safety of these designer drugs as well, in view of the fact that they are chemically closely related to MDMA and some have been shown to be 5-HT neurotoxins in animals.

Introduction

There is debate over the initial meaning of the term 'designer drug'. Some point out that it originally referred to drugs which were made for specific selected effects. However others claim that it is used to describe the private manufacture of synthetic analogues slightly different from parent compounds

that, by design, rendered them temporarily immune from the control of the Drug Enforcement Agency (DEA). In the past, changes to a chemical structure would allow a newly synthesized drug to escape regulation until the legal authorities discovered its existence. Since in 1986 the US Government (followed in 1997 by the EU) amended the Controlled Substances Act (CSA) with the "designer drug" legislation, today the DEA can take immediate action and schedule related substances.

Synthetic drugs with long histories of illicit use include amphetamines and lysergic acid diethylamide (LSD), while ecstasy (3,4-methylenedioxymethamphetamine; MDMA) and other drugs listed in Shulgin's Pihkal list (1991) have much shorter histories of illicit use. In the mid 1980's the term designer drug began to be used synonymously with dance or recreational drugs following the emergence of MDMA and other ring-substituted amphetamines in the recreational drug scene, although non-designer drugs such as cannabis and cocaine are also consumed in these settings. This is perhaps why the term designer drug nowadays lacks precision. As popularised by the lay press, the term is often used when referring to 'club drugs'. Club drugs are drugs that have become popular among teens and young adults at dance clubs and "raves", and not only include amphetamine analogues but non-designer drugs as well, such as flunitrazepam (Rohypnol®), gamma-hydroxybutyrate (GHB) and ketamine. However, because of the strong association in the literature with MDMA-like substances, in the present review the term designer drug will be used for synthetic drugs derived from amphetamine and methamphetamine: amphetamine analogues.

Amphetamine analogues are becoming increasing popular among adolescents and young adults. Of these, MDMA is unquestionably the most popular and extensively studied. Its use has shown a dramatic increase over recent years when use of other illicit drugs remained constant or decreased in the US (Strote et al., 2002; Johnston et al., 2002). The greatest concern with respect to the safety of MDMA and related drugs is the large body of animal literature suggesting that these drugs are selective neurotoxins. Studies have shown significant long-lasting neurochemical and morphological changes in serotonin (5-HT) neurones in response to MDMA, changes in dopamine (DA) neurones in response to amphetamine, and changes in both 5-HT and DA neurones in response to methamphetamine treatment. There is also growing concern about the potential manufacture of other and newer designer drugs sold as an alternative of MDMA, or which are added to MDMA tablets.

However, despite the popularity and considerable media attention, few detailed assessments of risks in humans exist. This may be due to the fact that previously, potential neurotoxic changes in the living human brain have only been identified using *indirect* methods. However, *in vivo* neuroimaging tools, such as positron emission tomography (PET), single photon emission computed tomography (SPECT), and several magnetic resonance (MR) imaging applications show great promise in *directly* identifying potential neurotoxic consequences of amphetamine designer drugs in the living human brain.

The goal of this article is to review the available neuroimaging data on potential neurotoxic effects on the 5-HT and DA system of several of the most

prevalent amphetamine analogues in order to delineate the risks these drugs engender in humans, focusing on MDMA.

Studying neurotoxicity

The four most widely used biological markers of 5-HT or DA neurotoxicity of amphetamine analogues as indicated by Kieven and Seiden (1989) in animals are:

— Long-lasting depletions in 5-HT or DA levels
— Decrease in 5-HT transporter (SERT) or DA transporter (DAT) densities
— Decreased activity of the synthetic enzymes for 5-HT or DA
— Alterations in neuronal morphology

All these criteria have been studied in animals treated with amphetamine analogues, amphetamine, and methamphetamine. In humans the following biological markers for 5-HT and DA neurotoxicity have been studied using in vivo neuroimaging techniques:

— Decrease in SERT or DAT
— Non-specific neuronal loss: decreases in levels of the neurometabolite *N*-acetylaspartate

Furthermore, if use of amphetamine or its analogues leads to brain pathology, this would result in impairments of functions in which 5-HT or DA play an important role (e.g., cognition, motor speed). Several studies have therefore attempted to investigate:

— An association between biological markers of neuronal damage and behavioural function

Although several neuroimaging studies have investigated whether use of amphetamine analogues and ther parent compound are associated with secondary, indirect, changes in post-synaptic 5-HT and DA receptor densities, brain microvasculature and cerebral glucose metabolic rate, this review will concentrate on the long-term effects of these drugs on the pre-synaptic 5-HT and DA system only.

In vivo neuroimaging techniques

PET and SPECT

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. The most advanced detecting 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 ^{99}mTc).

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 logistics and production of SPECT radiotracers, this technique is more widely available than PET.

SERT and DAT are structural elements of the 5-HT and DA neuron, respectively, and thought to be a reliable marker of the integrity of these neurons (Zhou et al., 1998; Scheffel and Ricaurte 1990; Villemagne et al., 1998). The use of these transporters as an index for neuronal integrity is indicated by findings of decreased brain SERT and DAT levels in animals treated with 5-HT and DA neurotoxins (Battaglia et al., 1987; Scheffel et al., 1998; Koda and Gibb, 1973; Seiden et al., 1976; Bakhit et al., 1981; Ricaurte et al., 1980).

Recently, PET and SPECT radioligands have been developed for neuroimaging of SERT and DAT in the human brain. Because animal studies already showed that MDMA-, and methamphetamine induced neurotoxicity is associated with loss of SERT, while amphetamine and methamphetamine-induced neurotoxicity is associated with loss of DAT, PET and SPECT are ideally suited for studying the potential neurotoxic effects of amphetamine analogues in the living human brain. However, because there are important features for a good *in vivo* tracer for these transporters, there are only a few radioligands (particularly with respect to the SERT) which fulfil at least some of the criteria and subsequently only several tracers have been used to investigate the effects of amphetamine designer drugs *in vivo.*

^{1}H MR Spectroscopy

Another way to investigate potential neurotoxic effects of amphetamine designer drugs, is by studying certain aspects of cerebral biochemistry using proton magnetic resonance spectroscopy (^{1}H MRS). Peaks of *N*-acetyl (NA) groups (primarily *N*-acetylaspartate, NAA), choline-containing compounds (Cho) and creatine plus phosphocreatine (Cr) can be obtained non-invasively. Determination of the myoinositol (MI) peak is also possible using short echo times. NAA is contained almost exclusively within neuronal cell bodies and axons (Howe et al., 1993), and considered a marker for neuronal loss or dysfunction (Urenjak et al., 1993; Higuchi et al., 1997), whereas MI is considered a possible marker for gliosis, reflecting ongoing repair processes.

Methylenedioxy analogues of amphetamine

Of the amphetamine analogues, the three methylenedioxy analogues of amphetamine, namely MDA (3,4-methylenedioxyamphetamine), its *N*-methyl analogue MDMA and *N*-ethyl analogue MDE (3,4-methylenedioxyethylamphetamine) are the best known, and collectively termed methylenedioxy analogues of amphetamine. They differ from amphetamine in that they have a methylenedioxy (-0-CH_2-0-) group attached to the aromatic ring (i.e., are

"ring-substituted"). MDMA and MDA were typical designer drugs until they were added to Schedule I of the US Controlled Drug Act in 1985, followed by MDE in 1986. MDA, MDMA and MDE have very similar effects in humans, but differ in potency, time of onset and duration of action (Hegadoren et al., 1999). They are typically used recreationally at "raves" because they enhance energy, endurance, sociability and sexual arousal, making them particularly popular among youngster.

Although data on the current prevalence of MDA and MDE are sparse, epidemiological studies have shown a dramatic increase in MDMA use over recent years. The prevalence of past year MDMA use among US college students rose from 2.8% to 4.7% between 1997 and 1999, an increase of 69% (Strote et al., 2002). In a 30 year longitudinal study at a large US college it was observed that weekly use of alcohol has remained stable, whereas most illicit drugs declined after a peak in 1978, except for MDMA which increased over the years (Pope et al., 2001). This study also showed that MDMA is the second most frequently tried illicit drug after marijuana. The Monitoring the Future Study (Johnston et al., 2002) has shown for 12th graders in the US that although last year MDMA use continued to increase (around 9% in 2001), the rate of increase began to fall off since 2001. In line with this, MDMA use has generally stabilised in the EU, although upward trends in MDMA use are still observed in some urban areas (EMCDDA 2001). However, the use of MDMA (and methamphetamine) has shown a huge increase in production and use in many Asian countries, like Hong Kong and Indonesia (Ahmad, 2002).

Animal studies

Almost more than two decades ago the first evidence emerged indicating that methylenedioxy analogues of amphetamine produced selective toxic effects on brain 5-HT neurons (Ricaurte et al., 1985). Of the methylenedioxy analogues, MDMA is by far the best studied. Dose-related reductions in brain markers of 5-HT axons, such as 5-HT and 5-hydroxyindolaceticacid (5-HIAA) (Commins et al., 1987; Schmidt et al., 1987a, 1986; Stone et al., 1986), SERT density (Battaglia et al., 1987; Commins et al., 1987) and the activity of tryptophan hydroxylase (Schmidt et al., 1987b; Stone, 1986) have consistently been reported in animals treated with MDMA. Similar observations have been made for MDA and MDE, even though MDE has been shown to be a less potent neurotoxin (Gibb, 1987; Johnson et al., 1986; Kamien et al., 1986; Stone et al., 1987).

MDMA-induced 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). 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 dopamine neurons. Interestingly, it has recently been shown that a closely spaced but low-dose regimen leads to severe dopaminergic neurotoxicity in addition to less pronounced serotonergic neurotoxicity in primates (Ricaurte et al., 2002). MDMA neurotoxicity was associated with increased vulnerability to motor dysfunction.

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., 1988; 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,b) 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 seem to 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 and Chappell, 1989).

Human studies

In humans, of all three methylenedioxy analogues, only the effects of MDMA on 5-HT neurons has been studied. Studies have been conducted in human MDE users, but these concerned brain microvasculature and cerebral glucose metabolic rate, which fall beyond the scope of this overview.

PET

Trans-1,2,3,5,6,10-β-hexahydro-6-[4-(methylthio)phenyl] pyrrolo-[2,1-α]isoquinoline ([^{11}C]McN5652) is the first PET radioligand successfully developed

in 1992 to label SERTs in the living human brain (Boja et al., 1992). *In vitro*, the active enantiomer (+)-[^{11}C]McN5652 binds selectively to SERTs, whereas the 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 in an MDMA-treated baboon. Mean radioactivity of (+)-[^{11}C]McN5652, but not (−)[^{11}C]McN5652, was reduced in all brain regions studied, ranging 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 post-mortem. Therefore, is 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., 1998a). The purpose of the study was to compare [^{11}C]McN5652 labelled SERT densities in human MDMA users with SERT densities in control subjects. Nine males and 4 females who reported previous use of MDMA were enrolled, along with 9 male and 6 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 SERT 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 showing 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.

SPECT

Using SPECT both the 5-HT and DA-system have been investigated in MDMA users.

5-HT system Since then, similar observations have been made using [^{123}I]β-CIT SPECT. The cocaine analogue 2β-carbomethoxy-3β-(4-iodophenyl)tropane (β-CIT) is presently the best studied and most used SPECT tracer for labelling of SERTs. [^{11}C]β-CIT binds with high affinity to both DAT and SERTs (Boja et al., 1991). The specific uptake of [^{123}I]β-CIT in the striatum is primarily associated with DAT, since it is blocked by the selective DA uptake inhibitor GBR 12,909 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, and possibly cerebral cortex) in which SERT densities 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 (Lew et al., 1996; Scheffel et al., 1992). 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 (Reneman et al., 2002a). 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 SERT densities obtained with autoradiography after sacrifice of the animal.

Several studies have recently been conducted using [^{123}I]β-CIT SPECT to study the effects of MDMA on human brain 5-HT neurons. 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 as compared to 10 well-matched controls. Reductions in binding inversely correlated with time since last MDMA use. No correlations were observed between [^{123}I]β-CIT binding ratios and a variety of neuropsychological measures. However, there are several problems associated with this study. Subjects were asked to abstain from psychoactive drugs for one week, whereas this was 3 weeks in the [^{11}C]McN5652 PET study (McCann et al., 1998a). 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 four 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 [^{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 neurons in several 5-HT rich brain regions. Three different subgroups of 54 MDMA users (moderate and heavy users, as well as ex-heavy 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 well matched for age, gender distribution and psychosocial factors. Overall tests for group and group by gender were significant, indicating a significant overall effect of MDMA use on [^{123}I]β-CIT binding which was significantly different for males and females. More specifically, [^{123}I]β-CIT binding ratios were significantly decreased in all brain regions studied in female, but not in male, heavy MDMA users, suggesting that females may be more susceptible than males to the neurotoxic effects of MDMA (Fig. 1). In line with this, a significant (negative) association was observed between extent of MDMA exposure with [^{123}I]β-CIT binding in females, but not in males. 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 SERT densities reported in MDMA users studied with [^{123}I]β-CIT SPECT by Semple and Reneman 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 SERT densities obtained with autoradiography in some brain regions (Reneman et al., 2002a). As for [^{11}C]McN5652 PET, also [^{123}I]β-CIT SPECT may lack adequate sensitivity to detect smaller MDMA-induced 5-HT lesions. The presence of SERTs in neocortical regions is well documented in human brain (Backstrom et al., 1989; Gurevich et al., 1996). There has however been some discussion in the literature about the ability of [^{123}I]β-CIT to bind to SERT in the cerebral cortex, since [^{123}I]β-CIT lacks selectivity for the SERT. Farde and co-workers (1994) have shown that [^{11}C]β-CIT uptake in monkey cortex could be displaced by 50% by citalopram, a selective SERT inhibitor, but not by GBR 12909, a selective dopamine transporter inhibitor. These findings are consistent with reports on binding of [$^{123/125}$I]β-CIT to SERTs in the neocortex of rats (Boja et al., 1992; Scheffel et al., 1992), suggesting that [^{123}I]β-CIT very likely binds to SERT in the cerebral cortex. Future studies should be conducted to validate the use of [^{123}I]β-CIT SPECT in detecting SERT reduction in the cerebral cortex.

DA-system Because tablets sold as "ecstasy" do often not only contain MDMA but other compounds well known to cause dopaminergic neurotoxicity such as (meth)amphetamine, and in view of a recent study suggesting that MDMA can lead to DA neurotoxicity in primates (Ricaurte et al., 2002), it is of interest to study potential DA neurotoxicity in humans.

In humans, one imaging study has been conducted to study the effects of MDMA on DA neurones. Using [^{123}I]β-CIT SPECT, Reneman and colleagues (2002c) compared striatal DAT densities in 29 sole MDMA users and compared them with DAT densities in 15 non-MDMA using control subjects. Subjects were scanned after an abstention period of at least 3 weeks. Striatal DAT densities were significantly increased (+13%) in MDMA users, after adjustment for age. These initial observations in humans suggest that sole use of MDMA is not related to dopaminergic neurotoxicity in humans, in contrast to data in primates showing severe DA neurotoxicity in primates exposed to an MDMA regimen modelled after one used by humans (Ricaurte et al., 2002).

^{1}H-MR Spectroscopy

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 (2002b) recently reported 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, as compared to 12 gender and age matched control subjects. 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 and that of Reneman, may be attributed in part to age-associated differences between both 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 discrepancy between the studies.

Obergriesser and colleagues (2001) compared hippocampal ^{1}H MR Spectroscopic Imaging (MRSI) data of 5 MDMA users with those of 5 controls, after a drug free period of at least 3 weeks. Hippocampal NAA levels and NAA/(Cr + Cho) did not differ between groups.

Linking biological markers of neuronal injury with behavioural function

By combining imaging studies with neuropsychological assessment it is possible to study links between neuronal loss, or brain damage, and cognitive function. 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, 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 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. Several studies have found an association between markers of neuronal injury and impaired cognitive function in MDMA users. Memory is of particular interest since several studies have found that recreational MDMA users display significant memory impairments, whereas their performance on other cognitive tests is generally normal (Parrott, 2000).

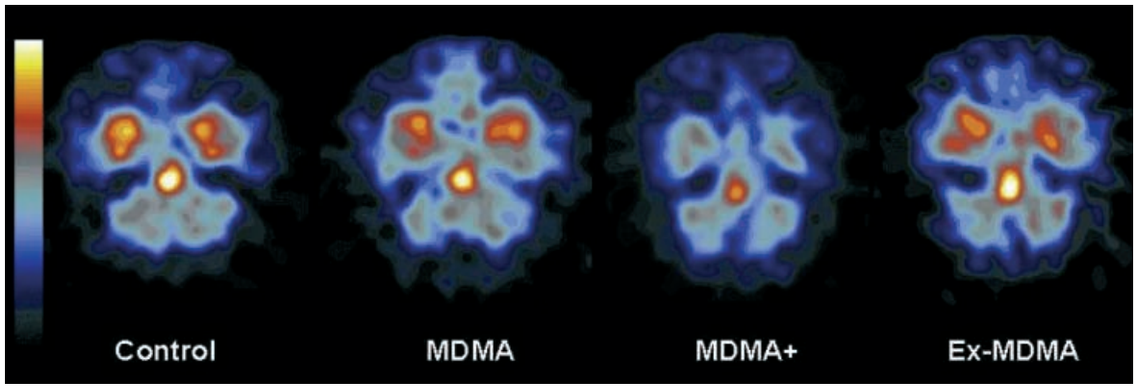

Fig. 1. [^{123}I]β-CIT SPECT images of a female control subject, a female moderate MDMA user (MDMA), a female heavy MDMA user (MDMA+) and a female ex-MDMA user (ex-MDMA). Transverse slices from the brain at the level of the midbrain. In the three images the level of [^{123}I]β-CIT activity is color encoded from low (black) to high (white) and scaled to the maximum in the slice of the control subject. The images show loss of 5-HT transporters in the midbrain of a female heavy MDMA user (from Reneman et al., 2001, with permission)

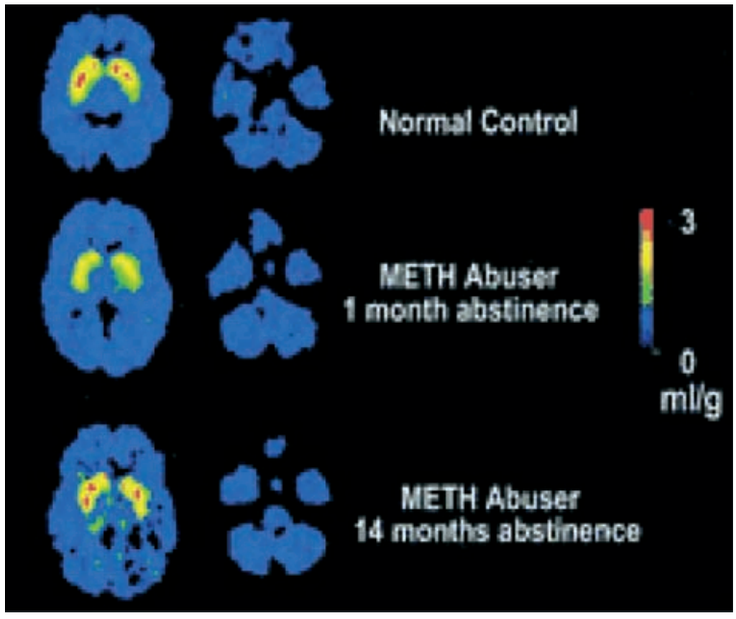

Fig. 2. Brain images of the distribution volume of [^{11}C]*d-threo*-methylphenidate in a control and a methamphetamine abuser. Images shown were obtained at the level of the striatum (images to the *left*) and the cerebellum (images to the *right*), and they are from a normal control and a methamphetamine abuser evaluated twice, during short and protracted abstinence. Notice the significant increases in binding in striatum in the methamphetamine abuser with protracted abstinence (from Volkow et al., 2001, with permission)

Reneman and colleagues (2001b) compared cortical [^{123}I]β-CIT labelled SERT densities in different groups of MDMA users: twenty-two recent MDMA users who did not use 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 were enrolled. In addition, memory was assessed using a word recall test (Rey Aditory Verbal Learning Test). These are the same subjects as in an earlier study (Reneman et al., 2001a), with exception of the moderate MDMA users. Reduced cortical SERT densities were observed in recent, but not ex-MDMA users. However, both recent as well as ex-MDMA users recalled significantly less words compared to 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. A similar observation was made by Semple (1999), although in the Semple study MDMA users and non-users did not differ in neuropsychological performance. In contrast, Reneman (2000a) observed a strong association between post-synaptic 5-HT_2-receptor densities and memory performance. These findings may 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 be long(er) lasting. It An alternative explanation may be that cognitive abnormalities in MDMA users are not related to 5-HT neurotoxicity, but rather DA-neurotoxicity (Ricaurte et al., 2002).

Finally, one other study has investigated the relation between brain damage and memory function (Reneman et al., 2001c). Again, RAVLT was used to study 8 abstinent MDMA users and 7 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 less words compared to 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 were conducted using small sample sizes with overlapping samples, they at least suggest an intriguing relationship between markers of brain damage and memory performance in MDMA users. However, they need be confirmed in a larger number of subjects.

Other amphetamine analogues currently used recreationally

In view of the fact that the number of potential synthetic amphetamine analogues that can be made and distributed are unlimited, only some can be discussed in this paper. The World Health Organization (2001) recently recommended for international control of the following analogues: 4-bromo-2,5-dimethoxyphenethylamine (2C-B), 4-methylthioamphetamine (4-MTA), and N-methyl-1-(1,3-benzodioxol 5-yl) 2-butanamine (MBDB), only the neurotoxic effects of 4-MTA and MBDB on brain 5-HT and DA neurones have been studied in animals. Administration of an acute dose of 4-MTA did not

affect 5-HT and 5-HIAA levels (Huang et al., 1992), suggesting that it has no 5-HT neurotoxic effects in rats. In contrast, MBDB, following a regimen of twice daily dosing for four days, produced significant decreases in 5-HT, 5-HIAA and [^{3}H]-paroxetine binding sites, although slightly less than MDMA. These data suggest that MBDB is a 5-HT neurotoxin, but may be less neurotoxic than MDMA (Johnson and Nichols, 1989).

Other amphetamine analogues that have been synthesized and used by humans are: para-methoxymethamphetamine (PMMA) and para-methoxyamphetamine (PMA). Both PMMA and PMA (80 mg/kg) have shown to produce comparable depletions of 5-HT, but these depletions are not as pronounced as those induced by a lower dose of MDMA (20 mg/kg), suggesting that PMMA and PMA are less potent than MDMA as a 5-HT neurotoxin (Steele et al., 1992). Striatal dopamine levels were unaffected by PMMA and PMA. On the other hand, 2,5-dimethoxy-4-methylamphetamine (DOM) does not cause significant loss of 5-HT uptake sites (McKenna et al., 1991). Neurotoxic effects of 2,5-dimethoxy-4-bromoamphetamine (DOB) have not been studied in animals.

5-HT neurotoxicity of none of the above mentioned analogues has been studied in humans.

Methamphetamine

Methamphetamine was first produced in the beginning of the 20th century by *N*-methyl substitution of amphetamine. Methamphetamine is a highly addictive drug (Woolverton et al., 1984), and its abuse has risen substantially. In several areas of the US one form of methamphetamine, crystal methamphetamine or "ice" grew in popularity in the 1980s. Its popularity declined again for a long period of time (0.4% in 1992), rising up again to 1.5% in 2001, an increase of 275% in the past decade (Johnston et al., 2002). The drug has now also become increasingly popular in Asian countries (Ahmad, 2002). In contrast, the average consumption of methamphetamines is still very limited in the EU (EMCDDA, 2001).

Animal studies

Methamphetamine administration to animals has been shown to produce long-lasting damage to DA and 5-HT neurones in animals (Koda and Gibb, 1973; Seiden et al., 1976; Bakhit et al., 1981; Ricaurte et al., 1980). After administration of methamphetamine animals develop long-lasting decreases in DA and 5-HT as well as their transporters (Seiden et al., 1976; Villemagne et al., 1998; Wagner et al., 1980). Although previous studies proposed that methamphetamine-induced DAT losses reflected irreversible terminal degeneration (Ricaurte and McCann, 1992b), recent studies in rodents (Friedman et al., 1998; Cass and Manning, 1999) and in nonhuman primates (Melega et al., 1997; Harvey et al., 2000) have revealed significant recovery with protracted

abstinence. There is also evidence that methamphetamine affects nonmonoaminergic cortical neurones (Commins and Seiden, 1986; Ryan et al., 1990).

Human studies

PET

Recently a number of imaging studies have reported on the effects of methamphetamine on DA neurones in the human brain. To my knowledge, SERT densities have up until now not been studied. Using PET, reductions in DAT have been demonstrated in human methamphetamine users using the DAT ligands [^{11}C]WIN-35,248 and [^{11}C]*d-threo*-methylphenidate (McCann et al., 1998b; Volkow et al., 2001a; Sekine et al., 2001). DAT losses varied from 24 to 30% in these studies.

Linking biological markers of neuronal injury with behavioural function
Because significant reductions in DAT occur both with age (6–7% per decade for the 20–80 year age span) (Volkow et al., 1996) a concern arises as to whether methamphetamine abusers will place them at risk for Parkinsonism as they age. Although the DAT loss reported in methamphetamine abusers is smaller than that reported in Parkinson's disease (36 and 71%) (Frost et al., 1993), it is nonetheless associated with reduced motor speed and impaired verbal learning (Volkow et al., 2001a) and the severity of persistent psychiatric symptoms (Sekine et al., 2001).

Because potential risk for Parkinsonism and cognitive decline in methamphetamine users likely depends in part on the reversibility of the changes induced by methamphetamine, Volkow and colleagues (2001b) recently investigated whether DAT losses in methamphetamine abusers recover with protracted abstinence. Brain DAT densities as obtained with [^{11}C]*d-threo*-methylphenidate PET in 5 methamphetamine abusers during short abstinence (6 months) were retested during protracted abstinence (12–17 months). Significant increases in DAT were observed with protracted abstinence, suggesting that DA terminals recover after methamphetamine-induced damage or that DAT losses short after methamphetamine reflect temporary adaptive changes (i.e., downregulation or internalisation). Because recovery of DAT after methamphetamine treatment in animals takes longer (6–9 months) than if they were due to DAT downregulation or internalisation, the authors concluded that likely DAT losses recover in methamphetamine users. However, a parallel improvement in function was not observed, since neuropsychological function did not recover with protracted abstinence.

^{1}H MR Spectroscopy

One study has investigated NAA levels in 26 abstinent amphetamine users (Ernst et al., 2000) and 24 healthy subjects without a history of drug abuse.

Methamphetamine subjects were scanned after last use of the drug more than 2 weeks earlier. In the basal ganglia and frontal white matter NAA levels were significantly reduced in methamphetamine users compared to controls, suggestive of neuronal damage in abstinent methamphetamine users. Frontal white matter NAA levels correlated inversely with previous extent of methamphetamine use. The methamphetamine users also showed significantly reduced total creatine in the basal ganglia and increased choline-containing compounds, as well as MI in the frontal grey matter.

Amphetamine

The parent compound of the above described analogues, amphetamine (alpha-methyl-phen-methylamine), is a synthetic stimulant first produced in 1887 and has been used clinically to treat doseases such as mild depression and abnormally hyperactive children. The drug can cause psychological dependence and is often sold and used illegally. Although illicit drug use of amphetamine is limited in the US, Scandinavian countries are the main market for injected amphetamine and the UK for non-injected amphetamine (EMCDDA, 2001). The highest consumption of amphetamine in EU is found in the UK (EMCDDA, 2001).

Animal studies

Amphetamine has been shown to be neurotoxic to DA neurones in animals. Administration of amphetamine to animals, including non-human primates, results in decreases in DA levels and DAT densities (Melega et al., 1996; Steranka, 1983; Steranka et al., 1980). PET studies in amphetamine treated monkeys have shown reductions in striatal [^{18}F]fluoro-L-DOPA uptake in vervet monkeys (Melega et al., 1997, 1996). Furthermore, studies on rat striatal DA system have established that chronic amphetamine exposure results in neurotoxicity characterised by swollen nerve terminals and degenerated axons (Ridley et al., 1982; Ryan et al., 1990).

Human studies

SPECT

At present only one imaging study has been conducted to study the effects of amphetamine on DA neurones in humans. Using [^{123}I]β-CIT SPECT, Reneman and colleagues (2002c) recently reported reduced striatal DAT densities (−20%) in 9 combined MDMA and amphetamine use when compared with 29 sole MDMA users, after adjustment for age. Subjects were scanned after an abstention period of at least 3 weeks. The effects of MDMA on DAT density were also investigated in this study, as discussed previously. These initial observations in humans suggest that the intentional use of

amphetamine by regular users of MDMA is associated with a reduction in nigrostriatal DA neurones.

Discussion and future studies

The goal of this article was to review available neuroimaging data in humans on 5-HT and DA neurotoxic effects of amphetamine analogues in humans, in addition to the parent compounds amphetamine and methamphetamine.

Of all amphetamine analogues, only the effects of MDMA and methamphetamine have been studied in human users. The reviewed neuroimaging studies provide suggestive evidence that users of MDMA are susceptible to 5-HT, but not DA neuronal damage, in which females may be more susceptible than males. The effects seem to be dose-related, possibly leading to functional impairments such as memory loss, reversible in some, but not all, brain regions. These results raise important questions as to the safety of MDMA by recreational users of this drug. However, before the findings of the presented studies can be validly used in prevention messages and clinical decision making, some of the results will have to be (re)confirmed in secondary studies, particularly concerning gender differences and (ir-) reversibility of MDMA's neurotoxic effects. In addition, neuroimaging reports suggest that users of amphetamine and methamphetamine may be at risk of developing reversible DA neurotoxicity, leading to long-term functional impairments such as reduced motor speed and impaired memory function. The effects of methamphetamine on 5-HT neurones have not been studied in the human brain.

Clearly, there are important methodological problems in studies conducted in humans, which are subject to ethical and methodological constraints (Curran, 2000; Kish, 2002). 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 designer drugs use and neurotoxicity. To definitively establish a causative link between observed neurotoxic changes and MDMA-, or (meth)amphetamine use, an experimental study design would be needed. Therefore, at this moment, it cannot be ascertained that humans are susceptible to MDMA-induced 5-HT injury or (meth)amphetamine-induced DA-injury. One possible approach would be to assess people both before and after they took the drug. Neuroimaging techniques may be very helpful in providing such longitudinal studies in human drug users.

Whereas both PET and SPECT have proven to be useful techniques in assessing the potential risk of MDMA and related drugs, more recently introduced MR imaging tools hold great promise (Reneman, 2001d), but will yet have to prove their potential to the field. Currently available radioligands may not have the requisite sensitivity to detect smaller neurotoxic lesions. Without doubt more selective radioligands for SERTs will be developed for PET or SPECT in the future that may be more sensitive in detecting SERT reductions. Although none of the currently available techniques is perfect, converging lines of evidence are needed (using combinations of different imaging

Table 1. Summary of neuroimaging studies investigating potential neurotoxic effects of amphetamine analogues and parent compounds in the human brain

Investigating	Biological marker	Technique used	Reference
MDMA			
Neuronal loss	SERT	[^{11}C]McN5652 PET	McCann (1998a)
		[^{123}I]β-CIT SPECT	Semple (1999), Reneman (2001a,b)
	DAT	[^{123}I]β-CIT SPECT	Reneman (2002c)
	NAA	^{1}H-MRS	Chang (1999)
Liking neuronal injury with functional impairment	5-HT transporter + memory	[^{123}I]β-CIT SPECT	Reneman (2001b)
	NAA + memory	^{1}H-MRS	Reneman (2002b)
Amphetamine			
Neuronal loss	DAT	[^{123}I]β-CIT SPECT	Reneman (2002c)
Methamphetamine			
Neuronal loss	DAT	[^{11}C]WIN-35,248 PET	McCann et al. (1998b)
			Sekine et al. (2001)
		[^{11}C]*d-threo*-methylphenidate	Volkow et al. (2001a,b)
Liking neuronal injury with functional impairment	DAT + motor speed and memory	[^{11}C]*d-threo*-methylphenidate	Volkow et al. (2001a)
	DAT + psychiatric symptoms	[^{11}C]WIN-35,248 PET	Sekine et al. (2001)

techniques) to make an adequate risk assessment of MDMA and related rugs. 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, post-mortem neurochemical and neuroanatomical studies can be conducted.

Future studies will have to find out 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 human beings (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. In addition, more studies should be conducted focusing on other systems than the serotonergic system to increase our understanding on the effects of MDMA and subsequent compensatory mechanisms in the brain. Finally, as pointed out previously, because users of amphetamine analogues are typically polydrug users, it will be important to assess drug-drug interactions in animals. It may well be that one drug enhances the potential neurotoxicity of another drug used at a later time (Hegadoren et al., 1999).

If indeed amphetamine analogues and (meth)amphetamine lead to 5-HT and/or DA neuronal injury the health implications may be considerable, in which they will be responsible for early or late neuropsychiatric morbidity. Neuroimaging techniques will greatly contribute to our understanding of amphetamine analogue'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.

In conclusion, based on animal data and suggestive evidence in humans, it seems likely that MDMA is neurotoxic to brain 5-HT neurones and (meth)amphetamine to brain DA neurones, resulting in long-lasting functional impairments such as memory loss. Although very little is known about the effects of other amphetamine analogues, there are important questions as to the safety of these designer drugs to the human brain, in view of the fact that they are chemically closely related to MDMA and (meth)amphetamine.

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Authors' address: L. Reneman, MD PhD, Department of Radiology, G1-214, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands, e-mail: l.reneman@amc.uva.nl

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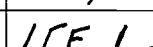
Differentiation of several errors in the same paragraph		There are many faults in this lane	r/ m/ aF i L i J
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