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REVIEW

Lost in translation: preclinical studies on 3,4-methylenedioxymethamphetamine provide information on mechanisms of action, but do not allow accurate prediction of adverse events in humans

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3,4-Methylenedioxymethamphetamine (MDMA) induces both acute adverse effects and long-term neurotoxic loss of brain 5-HT neurones in laboratory animals. However, when choosing doses, most preclinical studies have paid little attention to the pharmacokinetics of the drug in humans or animals. The recreational use of MDMA and current clinical investigations of the drug for therapeutic purposes demand better translational pharmacology to allow accurate risk assessment of its ability to induce adverse events. Recent pharmacokinetic studies on MDMA in animals and humans are reviewed and indicate that the risks following MDMA ingestion should be re-evaluated. Acute behavioural and body temperature changes result from rapid MDMA-induced monoamine release, whereas long-term neurotoxicity is primarily caused by metabolites of the drug. Therefore acute physiological changes in humans are fairly accurately mimicked in animals by appropriate dosing, although allometric dosing calculations have little value. Long-term changes require MDMA to be metabolized in a similar manner in experimental animals and humans. However, the rate of metabolism of MDMA and its major metabolites is slower in humans than rats or monkeys, potentially allowing endogenous neuroprotective mechanisms to function in a species specific manner. Furthermore acute hyperthermia in humans probably limits the chance of recreational users ingesting sufficient MDMA to produce neurotoxicity, unlike in the rat. MDMA also inhibits the major enzyme responsible for its metabolism in humans thereby also assisting in preventing neurotoxicity. These observations question whether MDMA alone produces long-term 5-HT neurotoxicity in human brain, although when taken in combination with other recreational drugs it may induce neurotoxicity.

LINKED ARTICLES

This article is commented on by Parrott, pp. 1518–1520 of this issue. To view this commentary visit http://dx.doi.org/10.1111/j.1476-5381.2012.01941.x and to view the the rebuttal by the authors (Green *et al.*, pp. 1521–1522 of this issue) visit http://dx.doi.org/10.1111/j.1476-5381.2012.01940.x

Abbreviations

AUC, area under curve; C_{max}, peak plasma concentration; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; HHA, 3,4-dihydroxyamphetamine; HHMA, 3,4-dihydroxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; *N*-Me-α-MeDA, *N*-methyl-α-methyldopamine; PKPD, pharmacokinetic pharmacodynamic integration; RNS, reactive nitrogen species; ROS, reactive oxygen species; T_{max}, time of drug peak; α-MeDA, α-methyldopamine

Introduction

The amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy') was first synthesized by the E. Merck company in Germany and patented in 1914 as a precursor for other compounds of possible commercial interest (Freudenmann *et al*., 2006). Its toxicology was studied in the 1950s by the US military (Hardman *et al*., 1973), presumably as a chemical warfare compound as its structure is similar not only to amphetamine but also mescaline. The first report of its psychoactive action in humans was by Shulgin and Nichols (1978) although the effects encountered are not detailed. Finally in the 1980s reports started to appear on its possible therapeutic properties with papers detailing its use in psychotherapy. It was reported to increase patient self-esteem and facilitate communication (Greer and Strassman, 1985; Greer and Tolbert, 1986; Grinspoon and Bakalar, 1986). However, in 1985 the US Drug Enforcement Administration classified MDMA as a Schedule 1 drug due to its high abuse potential, lack of clinical application, lack of accepted safety under medical supervision and evidence that its major metabolite 3,4-methylenedioxyamphetamine (MDA) induced degeneration of 5-hydroxytryptamine 5-HT neurones in brain (Ricaurte *et al*., 1985). Therefore possession of the drug was made illegal soon after in the UK and many other countries. This did not, however, curb its recreational use (it is generally referred to as 'ecstasy' or 'E') and its use became popular at 'rave' or 'techno' parties where ingestion was accompanied by dancing to heavily-mixed loud music, often with computer-generated videos and laser lights. A review of the epidemiological studies on the use of MDMA can be found in Green *et al*. (2003). More recently it has been reported that in the Netherlands less than 50% of confiscated ecstasy tablets contained MDMA as their primary component in 2009 compared with 90% in previous years (Brunt *et al*., 2011). In many of these tablets MDMA was substituted by other compounds and in 2009 mephedrone was found to be the most prevalent new designer drug to be misleadingly sold as ecstasy (EMCDDA, 2010).

Over the following 25 years a substantial number of studies have been conducted on the pharmacology of MDMA in laboratory animals. These investigations have provided detailed and valuable information on the mechanisms involved in producing the acute adverse effects of MDMA in experimental animals, particularly the body temperature changes (Docherty and Green, 2010) and the long-term neurotoxic effects of the drug in the brain (Green *et al*., 2003; Baumann and Rothman, 2009; Turillazzi *et al*., 2010).

However, as has recently been discussed elsewhere (Green *et al*., 2011), one fundamental aim of pharmacology is to perform preclinical studies that produce observations of use for determining the best clinical value of the drug and simultaneously evaluate any likely adverse effects or risks (Valentin *et al*., 2009), now often called translational pharmacology. In the case of MDMA such data can also help interpret the effects of the drug seen in recreational users. Furthermore, given the increasing interest in whether MDMA could have a beneficial role in treating psychiatric problems (Mithoefer *et al*., 2011), such data could be used to determine the overall safety profile of the drug.

Over 10 years ago Gijsman *et al*. (1999) expressed considerable disquiet about a study on the clinical pharmacology of MDMA by Vollenweider *et al*. (1998), suggesting that the dose of 1.7 $mg \cdot kg^{-1}$ that was administered to healthy volunteers was too 'close' to doses known to produce long-term neurotoxicity in experimental animals. Lieberman and Aghajanian (1999) reviewed both the safety aspects of the study and the ethical issue of giving experimental compounds to healthy subjects and, in general, found no grounds for concern. However, McCann and Ricaurte (2001) also expressed concern about the doses used in relation to those used in animal studies, concerns that Vollenweider *et al*. (1999; 2001) maintained were unwarranted. Further interest in the proposition that MDMA might have a therapeutic role has continued to be expressed. Grob *et al*. (1996) conducted some very preliminary studies on the possible therapeutic value of MDMA and more recently Sessa (2007) and Sessa and Nutt (2007) also raised the possibility that MDMA might be used as a tool in psychotherapy, although Green *et al*. (2008) did question some of their assumptions on both mechanisms of action and safety.

Consequently, information on whether preclinical data on MDMA can be translated to clinical research is now of particular value in order to design clinical studies that use doses that minimize the risk of short- or long-term adverse events. The recent encouraging results on the value of MDMA as an adjunct to psychotherapy for treating post-traumatic stress disorder (Mithoefer *et al*., 2011) suggests that further clinical studies with MDMA will follow. In addition, greater knowledge of the translatable value of preclinical studies to human drug ingestion will assist in evaluating possible harms in recreational users.

A basic requirement for successful translation is the availability of good pharmacokinetic data. When a drug is administered, its bioavailability, concentration – dose relationships, possible active metabolites and concentration-dependent plasma protein binding all influence its pharmacodynamic effect, and these factors may vary with species. All can confound the interpretation. It is rare for all the administered substance to produce the observed pharmacological effect and sometimes only a small fraction of the dose reaches the blood and the final target region. The rest may not be absorbed, or is metabolized before reaching the target organ (see Gabrielsson and Green, 2009 for a fuller discussion).

In this review we will argue that although many preclinical studies have provided important information on the general pharmacology and mechanisms of action of the drug, they may have limited translational value because no account has been taken of the very different pharmacokinetics of MDMA in rodents and humans. A further major problem is the inability to accurately quantify the doses of drug ingested or whether other recreational drugs were concomitantly taken in most of the published reports on the acute or long-term effects in recreational users. This makes it difficult to do 'reverse' translation of clinical observations to further assist interpretation of animal studies. Few studies on acute overdose in humans even supply plasma drug concentrations (Dowling *et al*., 1987; Henry *et al*., 1992) and post-mortem studies have limited value as the drug appears to redistribute after death (Elliott, 2005; De Letter *et al*., 2010).

Pharmacokinetic-pharmacodynamic integration

Pharmacokinetic pharmacodynamic integration (PKPD) or quantitative pharmacology is a well accepted technique integral to drug discovery and development by pharmaceutical companies to allow the most effective translation of preclinical studies to clinical application (Gabrielsson *et al.*, 2009; 2010; Valentin *et al.*, 2009). In a recent commentary Gabrielsson and Green (2009) argued that PKPD should now also be considered a key component of all preclinical pharmacology studies to allow the maximum information to be gained from every investigation and assist translation. They provided a list of the main components of PKPD that should be considered mandatory in any *in vivo* pharmacology study in order to maximize our ability to interpret results:

- assessment of drug exposure (plasma concentrations)
- metabolism of drug (are there active metabolites?)
- Are dose schedules relevant to the way the drug is used?
- plasma protein binding of the drug
- Is there a temporal mismatch between exposure and outcome measures?

In this review we will examine the available preclinical information on MDMA in the light of these proposals and ascertain where weaknesses in the current data limit our ability to translate the results into information that will be of benefit to the clinical investigator.

Assessment of drug exposure (plasma concentrations)

In the majority of preclinical studies on MDMA pharmacologists have related the response seen to the dose administered, but this approach ignores factors such as bioavailability, non-linear dose–plasma concentration relationships, active metabolites and plasma protein binding that may occur within or between species, and these all of these problems confound interpretation. Various approaches have been used to try and translate doses in rodents to those ingested in human recreational users. The simplest approach is a straight conversion of the dose per kilogram body weight (Sessa and Nutt, 2007). So, on the assumption that the average human weighs 70 kg, a dose of 5 mg·kg^{-1} to a rat is converted to a 350 mg dose in a human. Since recent studies have reported that most illegally obtained ecstasy tablets contain approximately 70 mg MDMA (Cole *et al.*, 2002; Morefield *et al.*, 2011), this suggests ingestion of about five tablets.

More sophisticated is the approach of Ricaurte and colleagues (McCann and Ricaurte, 2001; Mechan *et al.*, 2006) who used the allometric approach, which takes relative animal size into account (Mordenti and Chappell, 1989; Boxenbaum and D'Souza, 1990) and proposes the equation:

$$D_{human} = D_{animal} \cdot \left(\frac{W_{human}}{W_{animal}} \right)^{0.7}$$

where D is dose (mg), W is body weight (kg) and 0.7 the allometric constant. Use of the equation is claimed to provide calculation of equivalent doses in animals and humans. This equation suggests that a 5 mg·kg^{-1} dose to rats equates to 70 mg (1.0 mg·kg^{-1}) or around one ecstasy tablet in humans. However, Mordenti and Chappell (1989) stated that allometric scaling is often not relevant when metabolism of the drug produces active metabolites and, as will be discussed, this is a key matter when assessing both the acute and long-term effects of MDMA.

Of course, neither simple dose conversion nor allometric scaling allows for bioavailability, plasma protein binding or rates of metabolism. Better, although not ideal, is some basic indication of exposure such as the maximum plasma drug concentration following various doses of MDMA. Until recently information on exposure was difficult to obtain due to lack of published data; in humans partly because of perceived ethical constraints in performing dosing studies with an illegal drug, and in rats simply because the studies had never been performed.

In humans, ground breaking studies were performed by Rafael de la Torre and colleagues who examined the pharmacokinetics of MDMA in volunteers (Mas *et al.*, 1999; de la Torre *et al.*, 2000; 2004; Hernandez-Lopez *et al.*, 2002; Farré *et al.*, 2007). A more recent study by Kolbrich *et al.* (2008) confirmed and extended some of the earlier investigations. Arguably the most important observation was that in humans there is an increased gradient in the dose–plasma drug concentration slope with a fourfold increase in plasma concentration with only a twofold increase in dose from 1 to 2 mg·kg^{-1}. This is because in humans MDMA is metabolized by the polymorphic cytochrome P450 enzyme CYP2D6 (Tucker *et al.*, 1994) and MDMA is both metabolized by this enzyme and also inhibited by it (Tucker *et al.*, 1994), This results in the non-linear kinetics with inhibition occurring within 1 h (Yang *et al.*, 2006). In contrast, in rats there is an approximately linear relationship between dose and plasma concentration of the drug (Green *et al.*, 2009), although the response lacks linearity at higher doses due to saturation of hepatic clearance (de la Torre and Farré, 2004). Consequently the dose–plasma concentration curves are not markedly different between rats and humans at low doses (below 2.5 mg·kg^{-1}), but diverge rapidly at higher doses (Figure 1). Only at a dose of 5 mg·kg^{-1} did allometric scaling provide an accurate projection from rat to human exposure. At higher doses in humans, which could not be studied due to ethical constraints but are clearly used by some recreational users, the relationship breaks down totally because of auto-inhibition of CYP2D6 by MDMA (Figure 1).

The inhibition of CYP2D6 by MDMA in humans led to suggestions that this might account for the apparent poor relationship between reported doses ingested and severe acute adverse effects. Two human phenotypes of this enzyme exist, giving rise to fast metabolizers and poor metabolizers, the latter being deficient in the enzyme (5–9% of Caucasians) and thus at more at risk of acute toxicity (Tucker *et al.*, 1994). However, this is unlikely as all users of MDMA become effectively poor metabolizer phenotypes after ingestion of the first dose. What may well be a problem, however, is ingestion of the drug by those persons who take any other drug metabolized by CYP2D6, such as fluoxetine, which also inhibits the enzyme (Upreti and Eddington, 2007; Yubero-Lahoz *et al.*, 2011).

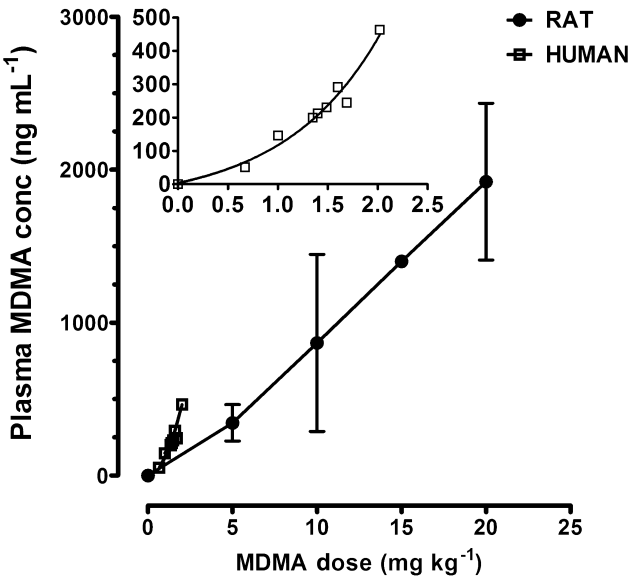

Figure 1

Plot of mean values of peak plasma MDMA concentration versus dose of MDMA administered taken from publications examining these parameters in rats and humans. Data in rats shown as mean value ± SEM of values from each study at that dose. Data in humans shows each separate study value obtained. Variance in these studies can be ascertained from the original papers. Insert figure shows human data in an expanded graph for clarity. Reproduced from Green *et al.* (2009) with permission of Springer-Verlag.

Examination of the recent detailed pharmacokinetic data provided by Baumann *et al.* (2009) in rats and Kolbrich *et al.* (2008) in humans using similar oral doses (2.0 and 1.6 mg·kg^{-1}, respectively) further supports the major difference between the kinetics of plasma MDMA in humans and rats (Table 1). There is a low peak plasma concentration (C_{max}) of MDMA in humans compared with rats and the time of this peak appears later (T_{max}). The $t_{1/2}$ in humans is over eight times longer than in rats, presumably because of slow metabolism of the parent drug, resulting in a larger area under the curve (AUC) in humans than rats. When the drug is given to rats via the commonly used i.p. route, the C_{max} following 2 mg·kg^{-1} is similar to that seen in humans at a dose of 1.6 mg·kg^{-1}. However, the T_{max} in rats is shortened further whereas $t_{1/2}$ and AUC are little altered (Table 1). This emphasizes the first main point that MDMA is both much more rapidly absorbed and also metabolized in rats compared with humans.

Metabolism of drug (are there active metabolites?)

The next major difference between humans and rats concerns the metabolic fate of MDMA (Figure 2). Not only is a single dose of MDMA cleared from plasma much more rapidly in rats than humans but the proportion of the metabolites formed differ. The rat metabolizes a significant proportion of MDMA by *N*-demethylation to MDA (23–34%; Baumann *et al.*, 2009) whereas this compound only forms a smaller portion (around 10%) of MDMA metabolism in humans (de la Torre and Farré, 2004). The fact that the proportion of MDMA converted to MDA is much smaller in the human is clearly confirmed by comparing pharmacokinetic data in human and rat (Table 2). A study in Dark Agouti (DA) rats (Valtier *et al.*, 2007) found an even greater proportion of MDMA was metabolized to MDA than in Sprague-Dawley rats as examined by Baumann *et al.* (2009). In an analogous fashion to the pharmacokinetic parameters seen with MDMA metabolism in rats and humans, the time to peak concentration (C_{max}) of MDA is much shorter in the rat, as is the half-life ($t_{1/2}$) of the drug in the plasma (Table 2).

What is important about these observations is that MDA has a similar pharmacology to MDMA producing both acute hyperthermia and neurotoxicity (Green *et al.*, 2003). It is thus known to be an acutely active pharmacological metabolite and also one that produces long-term neurotoxic damage at a lower dose than MDMA (Colado *et al.*, 1995).

The other major pathway of metabolism is *O*-demethylenation (Figure 2) and this also differs in rats and humans. In humans MDMA is metabolized by the polymorphic enzyme CYP2D6 (Tucker *et al.*, 1994) and because MDMA is both metabolized and inhibited by it (Tucker *et al.*, 1994), this results in the non-linear kinetics detailed earlier (de la Torre *et al.*, 2000). Although non-linear kinetics are also observed in rats (Baumann *et al.*, 2009) and squirrel monkeys (Mueller *et al.*, 2009), this effect is not marked in rats and the enhanced plasma drug concentration at higher doses may be due in part to hepatic saturation (de la Torre and Farré, 2004).

Although CYP2D6 is not present in rats, they possess a homologous but distinct cytochrome enzyme CYP2D1 (Malpass *et al.*, 1999; Maurer *et al.*, 2000). A study in DA rats demonstrated that MDMA was metabolized by CYP2D1 in this strain and also that female DA rats were deficient in this enzyme, compared with males (Kumagai *et al.*, 1994). Females can therefore be used as a model of the human poor-metabolizer phenotype. Administration of MDMA to both sexes resulted in females having both higher plasma concentrations of MDMA post-injection and also a greater hyperthermic response at all doses examined (Colado *et al.*, 1995), which also illustrates the fact that it is MDMA itself that induces the hyperthermic response. Although rats can display either a hyperthermic or hypothermic response to MDMA administration, this appears to be primarily related to the surrounding ambient temperature and also whether animals are grouped or individually housed (Docherty and Green, 2010). However, in contrast, MDMA administration to monkeys (Von Huben *et al.*, 2007) and humans (Freedman *et al.*, 2005) results in hyperthermia in low, normal or high ambient temperature conditions. The temperature response in rats (both hyperthermia and hypothermia) has been shown to be principally due to MDMA-induced dopamine release in the brain (Docherty and Green, 2010). The locomotor response is probably also largely due to dopamine and 5-HT release, whereas the appearance of the serotonin behavioural syndrome clearly results from 5-HT release alone (Green *et al.*, 2003 Baumann and Rothman, 2009). Other acute endocrine and cardiovascular effects are also generally considered to be induced by the acute release of monoamines

Table 1

Pharmacokinetic constants for plasma MDMA after administration of MDMA to rats or humans

Measure	Human MDMA: 1.6 mg·kg^{-1} po [Kolbrich *et al.*]	Rat MDMA: 2 mg·kg^{-1} po [Baumann *et al.*]	Rat MDMA: 2 mg·kg^{-1} i.p. [Baumann *et al.*]
Cmax (ng mL^{-1})	292 ± 76	46 ± 15	210 ± 108
Tmax (h)	2.4 ± 0.6	0.56 ± 0.31	0.14 ± 0.08
$t_{1/2}$ (h)	8.1 ± 2.1	0.77 ± 0.11	0.80 ± 0.16
AUC (h.ng mL^{-1})	3485 ± 760	61 ± 42	163 ± 56

Data taken from Baumann *et al.* (2009) and Kolbrich *et al.* (2008) as shown.

and resultant interactions of these monoamines with their receptors (Baumann and Rothman, 2009).

As stated earlier, MDMA is *N*-demethylated to MDA (Figure 2). MDMA and MDA are *O*-demethylenated respectively to *N*-methyl-α-methyldopamine (*N*-Me-α-MeDA), also called 3,4-dihydroxymethamphetamine (HHMA) and α-methyldopamine (α-MeDA), also called 3,4-dihydroxyamphetamine (HHA) (Lim and Foltz, 1988; Kumagai *et al.*, 1994). These catechols can undergo oxidation to *o*-quinones that are highly redox-active molecules and produce free radicals (Figure 2), reactive oxygen species (ROS) or reactive nitrogen species (RNS) (Green *et al.*, 2003; de la Torre and Farré, 2004). It is important to note that further metabolism of these compounds is much more rapid in rats than humans (Table 3).

N-Me-α-MeDA, α-MeDA and the *o*-quinones may be conjugated with glutathione to form a glutathionyl adduct (Hiramatsu *et al.*, 1990; Bai *et al.*, 1999; 2001). This conjugate remains redox-active, being readily oxidized to the quinone thioether (Figure 2). These metabolic pathways have been reviewed elsewhere (Easton *et al.*, 2003; Easton and Marsden, 2006; de la Torre and Farré, 2004; Capela *et al.*, 2006) and many of the metabolites are clearly pharmacologically active. They will be discussed further in sections on neurotoxicity.

It should also be mentioned that MDMA is a chiral compound normally used (ingested in humans, injected in rats) as a racemate. The (+)-S-enantiomer is more pharmacologically active than the (–)-R-enantiomer (see Green *et al.*, 2003) and studies in rats have indicated that the drug also undergoes stereoselective disposition, with the (+)-S-enantiomer having a shorter half-life than the (–)-R-enantiomer (Fitzgerald *et al.*, 1990). The more active *S*-enantiomer has a reduced area under curve (AUC) and $t_{1/2}$ than (*R*)-MDMA (Fallon *et al.*, 1999). In addition, preferences for S-enantiomer metabolites were observed for DHMA sulphation, but not for HMMA sulphation (Schwaninger *et al.*, 2012). However, these differences are probably not important in the overall pharmacology and pharmacokinetics of the racemic drug.

Are dose schedules relevant to way drug is used?

The normal route of drug administration is often ignored by experimental pharmacologists, and never more so than by those investigating MDMA. Generally the drug is ingested orally by recreational users, although cases of intravenous injection have been reported. In contrast, the majority of preclinical studies in rats have given the drug by the i.p. route. Although this may not be an important weakness in studies examining mechanisms of action of the drug, it markedly weakens the translational value of any data obtained. Nowhere is this illustrated better than the study of Baumann *et al.* (2009) where it was shown that administering the drug i.p. rather than orally increased the C_{max} by approximately fourfold and also increased T_{max} fourfold and the AUC threefold. If acute adverse effects are due to the parent drug and long-term toxicity results from the metabolism of MDMA and formation of neurotoxic metabolites (see later) these differences could have major consequences to our ability to interpret changes seen in animals to putative acute and long-term changes in humans.

Plasma protein binding of the drug

The degree by which a drug binds to plasma proteins determines the amount of unbound ('free') drug and this is the concentration of pharmacologically active drug. It is often not appreciated that this fraction can vary markedly not only between humans and rats, but between different species of laboratory animal (see Figure 10 in Gabrielsson *et al.*, 2010). There has been only one study on the plasma protein binding of MDMA, and that is in dogs (Garrett *et al.*, 1991), so no studies to date appear to have factored this variable into the investigation. Such information could be vital to designing more accurate dosing schedules and if there are marked differences between the protein binding of MDMA in rats and humans redrawing Figure 1 with the substitution of plasma unbound MDMA concentration might give a very different plot.

Is there a temporal mismatch between exposure and outcome measures?

Acute pharmacodynamic effects

All available data suggest that there is a close relationship between the concentration of available MDMA and the acute pharmacological responses to the drug. The acute body

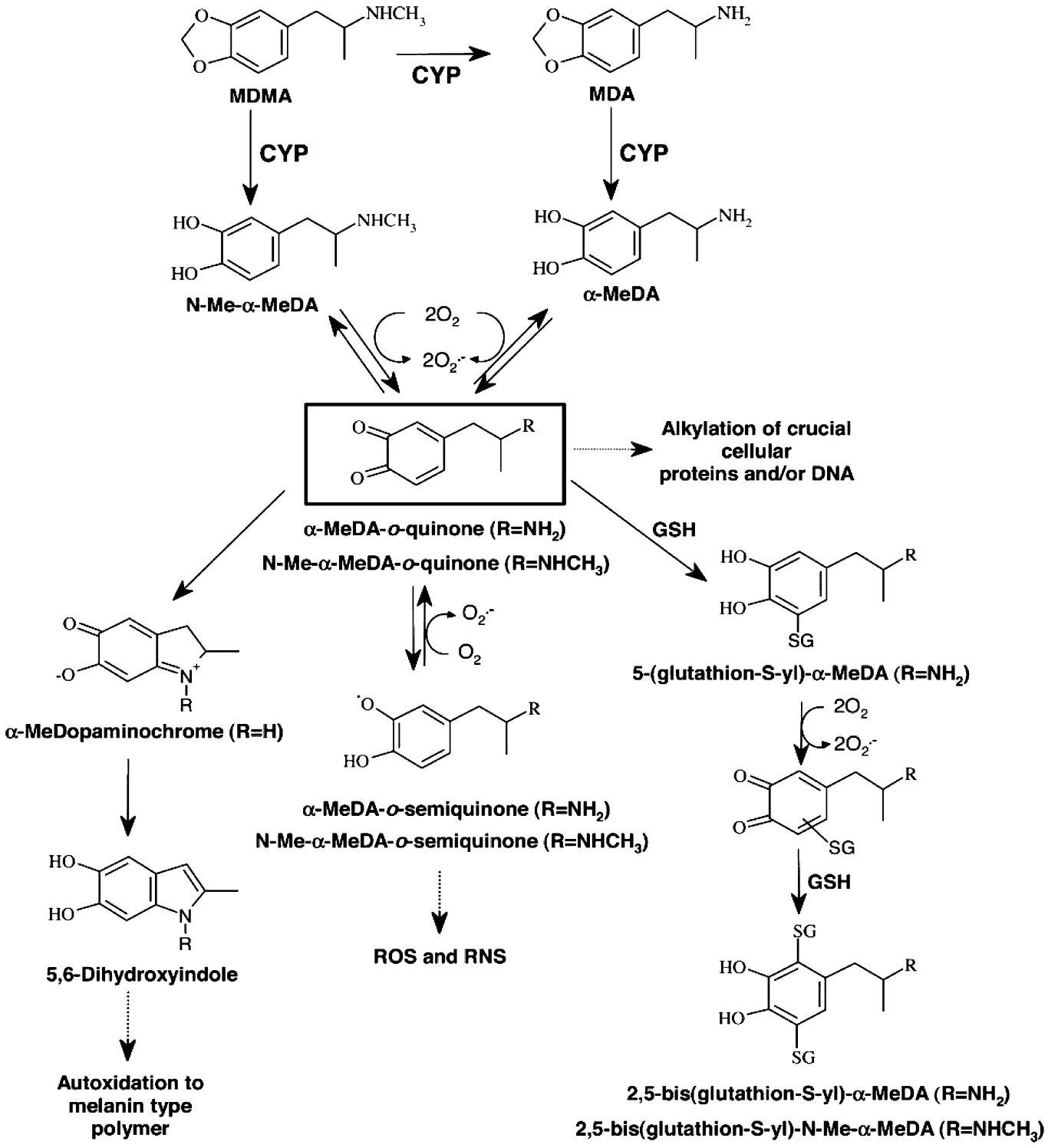

Figure 2

Proposed pathway for MDMA metabolism to neurotoxic metabolites. MDMA can undergo *N*-demethylation to MDA. Cytochrome P450 (CYP) enzymes also mediate demethylenation of MDMA and MDA to *N*-Me-α-MeDA and α-MeDA respectively. The catechols are readily oxidized to the corresponding *o*-quinones, which can enter redox cycles with their semiquinone radicals, leading to formation of ROS and RNS. On cyclization, *o*-quinones give rise to the formation of aminochromes and related compounds, such as 5,6-dihydroxyindoles, which can undergo further oxidation and polymerization to form brown or black insoluble pigments of melanin type. Alternatively, *o*-quinones can react readily with GSH to form the corresponding GSH conjugates like 5-GSH-α-MeDA. Reproduced from Capela *et al.* (2006) with permission of the American Society for Pharmacology and Experimental Therapeutics.

Table 2

Pharmacokinetic constants for plasma MDA after administration of MDMA to rats or humans

Measure	Human MDMA:1.6 mg·kg^{-1} po [Kolbrich *et al.*]	Rat MDMA:2 mg·kg^{-1} po [Baumann *et al.*]	Rat MDMA: 2 mg·kg^{-1} i.p. [Baumann *et al.*]
Cmax (ng mL^{-1})	13.8 ± 3.8	13 ± 4	21 ± 13
Tmax (h)	7.6 ± 2.6	1 ± 0.5	0.75 ± 0.29
$t_{1/2}$ (h)	12.3 ± 3	ND	2.51 ± 1.01
MDMA/MDA	20.8	3.5	10

Data taken from Baumann *et al.* (2009) and Kolbrich *et al.* (2008) as shown.

Table 3

Pharmacokinetic constants for plasma MDMA metabolites after administration of MDMA to rats or humans

Measure	Human MDMA:1.6 mg·kg^{-1} po [Kolbrich *et al.*]	Rat MDMA:2 mg·kg^{-1} po [Baumann *et al.*]	Rat MDMA: 2 mg·kg^{-1} i.p. [Baumann *et al.*]
HMMA			
Cmax (ng mL^{-1})	173.5 ± 66.3	141 ± 59	186 ± 88
Tmax (h)	1.9 ± 0.5	3.86 ± 0.75	4.25 ± 0.87
$t_{1/2}$ (h)	13.4 ± 2.7	2.57 ± 0.88	2.25 ± 0.23
AUC (h.ng mL^{-1})	2345 ± 670	702 ± 257	926 ± 417
HMA			
Cmax (ng mL^{-1})	3.9 ± 0.9	15 ± 2	13 ± 4
Tmax (h)	15.1 ± 7.9	4.00 ± 0.88	4.00 ± 0.88
$t_{1/2}$ (h)	122.3 ± 157.7	ND	ND
AUC (h.ng mL^{-1})	603 ± 695	85 ± 12	85 ± 12

Data taken from Baumann *et al.* (2009) and Kolbrich *et al.* (2008) as shown.

temperature response in rats shows a normal sigmoid dose–response relationship (Colado *et al.*, 1995) and there is a close temporal relationship between the increase in monoamine release (Mechan *et al.*, 2002) and the duration of the increase in plasma drug concentration (Baumann *et al.*, 2009) both of which are fairly short lasting (1–2 h). Locomotor activity and body temperature change also show a similarly brief response in normothermic room conditions (Mechan *et al.*, 2002; Rodsiri *et al.*, 2011). As many of the adverse effects also relate to hyperthermia it is reasonable to assume that either the parent drug, or the immediate metabolite MDA, which has a similar pharmacology (Colado *et al.*, 1995; Green *et al.*, 2003), are responsible.

The fact that plasma MDMA concentrations and functional outcomes are similar in rats and humans at low dose administration (1–2 mg·kg^{-1}) (Baumann and Rothman, 2009) suggests that most acute consequences in humans can be modelled in rats. The problem in translation is therefore related predominantly to that of extrapolating from rats when higher doses are administered, particularly given the shorter $t_{1/2}$ of MDMA in rats (and squirrel monkeys) than humans. We suggest that those scientists using rats to model most accurately the acute effects of MDMA in humans should consider altering the drug administration to ensure a longer duration of action, for example by use of slow infusion, implantation of an osmotic mini-pump or consecutive drug administration over time (essentially 'binge-style dosing'). This last approach, however, probably adds in the complication of increasing animal handling stress. Use of osmotic minipumps is also problematic in that it requires an operative procedure to implant the device, which may alter the response under investigation (Gabrielsson and Green, 2009).

Long-term neurotoxicity

In the case of neurotoxicity there is clearly a substantial temporal mismatch between drug exposure and outcome measures as neurotoxicity only becomes apparent after several days and is then detectable for several months (rats) or 2–3 years (monkeys) (see Green *et al.*, 2003; Baumann and Rothman, 2009). Before considering what MDMA pharmacokinetics might tell us about the mechanisms of neurotoxicity and discussing whether findings in laboratory animals might translate into long-term neurotoxicity problems in humans the postulated mechanisms involved must first be considered.

The first, and vital, point to be made is that MDMA injected into the brain does not cause neurotoxic damage. This was shown in a preliminary study by Paris and Cunning-

ham (1992) and confirmed and extended in a major study that measured MDMA concentration in the brain following a peripheral neurotoxic dose and matched it when using direct injection of the drug into the hippocampus (Esteban *et al.*, 2001). Collectively these data suggest that the drug is probably metabolized peripherally to an active metabolite or metabolites that is, or are, responsible for the damage. However, alternative mechanisms have been proposed.

Sprague *et al.* (1998) suggested that the dopamine released in the brain by systemic MDMA administration was transported into 5-HT nerve terminals, where it is oxidized to produce free radicals that produce the long-term damage. However, against this hypothesis stands the observation that depletion of cerebral dopamine content with α-methyl-*p*-tyrosine while keeping the animals normothermic (Yuan *et al.*, 2002), or enhancing dopamine content by administering L-DOPA (Colado *et al.*, 1999) both failed to alter MDMA-induced neurotoxicity. In addition, the question arises as to how dopamine can be involved in the MDMA-induced neurotoxicity in brain areas that are sparsely innervated by dopaminergic neurones.

Although MDMA injected directly into the brain does not produce neurotoxicity it does induce monoamine release (Esteban *et al.*, 2001). Therefore dopamine would have been released following central MDMA injection. If the released dopamine initiated the production of neurotoxic metabolites in 5-HT nerve endings then resultant neurotoxic damage should have been observed. The fact that it was not also argues against dopamine playing a key role in the loss of 5-HT nerve terminals.

Breier *et al.* (2006) reported that MDMA increased the extracellular tyrosine concentration in the striatum and hippocampus, and demonstrated that this tyrosine could be converted to DOPA and dopamine via a tyrosine hydroxylase-independent mechanism. They suggested that that this increased dopamine concentration was primarily responsible for MDMA-induced 5-HT neurotoxicity and supported this hypothesis by demonstrating that infused tyrosine enhanced MDMA-induced 5-HT neuronal damage. Both Gonĩ-Allo *et al.* (2008a) and Rodsiri *et al.* (2010) confirmed that systemic MDMA administration increased the tyrosine concentration in the hippocampus and cortex. However, Gonĩ-Allo *et al.* (2008a) also reported that MDMA administration increased the serum tyrosine concentration, which suggested that the rise in brain tyrosine might be the consequence of a preceding peripheral elevation of the amino acid. Other observations indicated that increased cerebral tyrosine enhanced the MDMA-induced temperature response and tyrosine depletion attenuated it (Gonĩ-Allo *et al.* (2008a); Rodsiri *et al.*, 2010). Breier *et al.* (2006) did not measure the temperature of the animals infused with tyrosine, so it is possible that by infusing tyrosine they enhanced the hyperthermic response of rats to MDMA, and there is extensive evidence that such an in increase in body temperature would increase the resultant neurotoxicity (Green *et al.*, 2004).

In conclusion, although a recent review has continued to support the hypothesis that dopamine release may play a pivotal role in MDMA-induced neurotoxicity (Puerta *et al.*, 2009) the supporting evidence remains weak.

Considerably more compelling is the proposal that it is metabolites formed by the peripheral metabolism of MDMA that cause neurotoxicity. This seems the most parsimonious explanation for the observation that MDMA administration directly into the brain does not induce neurotoxicity (Paris and Cunningham, 1992; Esteban *et al.*, 2001) and over the last few years has received considerable experimental support. The catechol metabolites can undergo oxidation to *o*-quinones that are highly redox-active molecules and produce free reactive oxygen or nitrogen species (ROS or RNS) radicals (Figure 2). *N*-Me-α-MeDA, α-MeDA and the *o*-quinones may be conjugated with glutathione to form a glutathionyl adduct (Hiramatsu *et al.*, 1990; Bai *et al.*, 1999; 2001). This glutathione conjugate remains redox-active, being readily oxidized to the quinone thioether. MDMA metabolism therefore leads to the formation of reactive intermediates (ROS and RNS), and/or toxic oxidation products, which may be responsible for the toxicity exerted by MDMA (Capela *et al.*, 2009; Song *et al.*, 2010). This proposal is supported by the observation that administration of the free radical trapping agent α-phenyl-*N*-tert-butyl nitrone (PBN) attenuated the long-term loss of 5-HT in the rat brain induced by MDMA (Colado and Green, 1995; Colado *et al.*, 1997; Yeh, 1999).

Although neurotoxicity can occur in rats following MDMA doses that do not induce an acute hyperthermia (O'Shea *et al.*, 1998), preventing the acute hyperthermia attenuates long-term neurotoxicity (Colado *et al.*, 1998) and increasing the ambient temperature increases the severity of the MDMA-induced neurotoxicity (Malberg and Seiden, 1998; Green *et al.*, 2004). These findings demonstrate that acute hyperthermia enhances long-term neurotoxicity in rodents. Free radical formation in the brain produced by MDMA administration was also increased by hyperthermia (Colado *et al.*, 1997), which is consistent with the fact that ischaemia-induced free radical formation is also influenced by body temperature (Globus *et al.*, 1995). These findings are linked to MDMA metabolism by two key observations. First, Gonĩ-Allo *et al.* (2008b) found that MDMA metabolism was inhibited when rats were housed in cool conditions, the plasma MDMA concentration being enhanced whereas the concentration of the major metabolites was reduced. In contrast, housing the animals at 30°C at the time of MDMA administration greatly enhanced the concentration of MDMA metabolites in the plasma. Second, in an *in vitro* study, Capela *et al.* (2006) observed that the neurotoxic effects of several MDMA metabolites on rat cortical neurones were attenuated by the presence of free radical scavengers and enhanced by hyperthermic conditions.

The presence of neurotoxic metabolites in brain (Jones *et al.*, 2005) further strengthens the view that these metabolites are responsible for cell death, as does the finding that administration of entacapone, a catechol O-methyl transferase inhibitor, to increase the concentration of HHMA and HHA exacerbated neurotoxic damage (Gonĩ-Allo *et al.*, 2008b). Mueller *et al.* (2009) have argued that the plasma MDMA concentration is a better indicator of damage than HHMA, but we would argue that this is not a surprising as MDMA is the initiator of the neurotoxic process while plasma concentrations of metabolites are in dynamic flux. Presumably several metabolites are involved in neurotoxicity and measurement of one or two, either conjugated or not, is unlikely to reflect their action on cell death.

Finally it is notable that mice metabolize MDMA differently to rats (Colado *et al.*, 2001; 2004; de la Torre and Farré, 2004; Escobedo *et al.*, 2005; Easton and Marsden, 2006; de la Torre *et al.*, 2009) and in mice MDMA produces dopaminergic neurotoxicity rather than serotonergic neurotoxicity (Logan *et al.*, 1988; Colado *et al.*, 2001; O'Shea *et al.*, 2001). This again points to MDMA metabolites rather than MDMA itself being the causative factor in neurotoxic damage to 5-HT neurones.

Can we extrapolate acute and long-term toxicity changes in animals to effects in human subjects?

Acute effects of MDMA

Many studies have shown that administration of a single dose of MDMA to rats produces a similar spectrum of physiological changes to that seen after a single ingestion of the drug by human subjects. The responses observed in animals include changes in body temperature, endocrine systems and cardiovascular effects (for details see Green *et al.*, 2003; Baumann and Rothman, 2009; Docherty and Green, 2010). However, the short $t_{1/2}$ in rats compared with humans means that many of these changes are relatively transient and a more sustained dosing period in the rat might give a better translational model of the effects of a single dose in humans. It is notable that three low doses (2 mg·kg^{-1}) of MDMA given to a rat over 6 h gives a much higher maximum hyperthermic response than seen when giving a similar (5 mg·kg^{-1}) single dose of the drug (Green *et al.*, 2005) despite the fact that the short half-life means that drug accumulation would not have occurred using the dosing interval employed. We might therefore conclude that prolonged exposure to the drug gives a more severe acute response.

Interestingly, the conditions experienced by young people in dance clubs of loud noise, hot, crowded rooms and lack of fluids for drinking had been shown around 50 years earlier to enhance the toxic effects of amphetamine in rodents (Gunn and Gurd, 1940; Chance, 1946). The phenomenon was called 'aggregation toxicity' and the pernicious influence of such conditions on the acute effects of MDMA has also been demonstrated (Fantegrossi *et al.*, 2003). Consequently the housing conditions of rats must be considered when trying to translate data to human studies. Aggregation may be relevant to dance club conditions (Parrott, 2006), but not when the drug is being investigated for therapeutic application.

A considerable number of studies have been conducted on whether acute ingestion of the drug produces psychomotor, cognitive or mood change or other short-term effects in humans. It is beyond the scope of this article to review such studies. We would merely suggest that in this instance preclinical studies should parallel the slow metabolism of the drug in humans if they wish to 'translate' to the clinical data currently available.

Long-term neurotoxic effects of MDMA

This is the cardinal question if MDMA is to be used as a therapeutic agent. It has been previously suggested by many authors that recreational MDMA ingestion might lead to neurotoxic damage to 5-HT neurones in the brain of human users and that this damage might be initially occult and only become functionally apparent after several years. Indeed one of us supported this contention many years ago (Green and Goodwin, 1996). This concern spread to unease by some investigators (Gijsman *et al.*, 1999; McCann and Ricaurte, 2001) that the studies of Vollenweider and colleagues (Vollenweider *et al.*, 1998; Liechti and Vollenweider, 2000a,b) should not have been undertaken. The contention was that the clinical studies on the pharmacology of the drug were using doses (1.7 mg·kg^{-1}) that could produce long-term adverse effects. Vollenweider *et al.* (2001) in a firm rebuttal of this contention pointed out that the dose used was based on a careful review of the literature and was in the lowest range required to reliably produce psychoactive effects (Downing, 1986; Greer and Tolbert, 1986; Grob *et al.*, 1996, 1998; Vollenweider *et al.*, 1999). Similar doses have now been used by Mithoefer *et al.* (2011) as an adjunct to psychotherapy in treating post-traumatic stress disorder with initially encouraging therapeutic results.

However, the apparent safety of low dose administration of the drug in carefully controlled conditions does not answer the question as to whether the drug produces neurotoxicity in recreational drug users, many of whom routinely ingest around three times the 'therapeutic' dose or use binge administration techniques, often in hot crowded conditions. Considerable efforts have been made to see whether volunteers who have ingested ecstasy have impaired neurophysiological or neuropsychological function. However, overall the evidence for impairment remains weak and controversial, which is surprising since it has been estimated that around 500 000 young people ingest MDMA on a weekly basis and this has been occuring over the last 25 years, although the population of the group obviously changes over time (Green, 2004). Crucially a major weakness of all studies examining either acute or long-term effects is a lack of information on the exact dose ingested. The drug has been obtained illegally and therefore no reliable information is available on either the purity or the dose consumed. Even dose frequency is dependent on information supplied by the user, which too may be inaccurate.

A further, and major, complication is that most recreational drug users take more than one type of legal (alcohol, nicotine) or illicit (cannabis, cocaine, opioids) drug and often at the same time (Morefield *et al.*, 2011). The ingested tablet is also often contaminated with additional active ingredients including other amphetamine derivatives. So the question arises as to whether any apparent brain abnormality is due to MDMA, another drug, or the combination of MDMA and other drugs? The complexity of this point is the subject of a very recent review (Mohamed *et al.*, 2011).

This review is only examining the pharmacokinetic evidence as to whether MDMA alone is likely to produce 5-HT neurotoxicity and our suggestion is that it is unlikely for two main reasons. The first is that the evidence is heavily weighted to the contention that it is MDMA metabolites, formed peripherally and then transported into the brain that cause neurotoxic damage in rats, squirrel monkeys and baboons and the available pharmacokinetic data suggests that the formation of these metabolites is very different in

Table 4

Time to peak plasma concentration (T_{max})and $t_{1/2}$ of MDMA in several species, data taken from Baumann *et al.* (2009), Mechan *et al.* (2006), Mueller *et al.* (2011) and Kolbrich *et al.* (2008)

Measure	Rat	Squirrel Monkey	Baboon	Human
T_{max} (h)	0.56	1.13	0	2.4
$t_{1/2}$ (h)	0.77	2.6	0	8.1

humans to these other species. Table 4 demonstrates that in the human the time to C_{max} is at least twice that seen in monkeys, baboons and rats and importantly that the clearance as indicated by $t_{1/2}$ (which presumably primarily reflects the rate of metabolism) is 10 times slower in the human than the rat and at least three times slower than monkey. The baboon is of particular interest in that MDMA is very rapidly metabolized to HMMA and HHMA. Both of these metabolites, but not MDMA, could be detected in plasma (Mueller *et al.*, 2011) and MDMA-induced neurotoxic damage has been reported to occur in this species (Scheffel *et al.*, 1998; Szabo *et al.*, 2002).

We therefore propose that it is principally the fast metabolic clearance of MDMA that induces neurotoxic damage in laboratory animals. The rapid formation of neurotoxic metabolites overwhelms the ability of the endogenous radical trapping agents such as ascorbic acid, catalase and superoxide dismutase to inactivate the reactive species being formed. This leads to neuronal damage. In humans the metabolism of MDMA is much slower so the toxic metabolites are formed at a sufficiently slow rate, as indicated by the pharmacokinetic measures detailed in this review, to allow endogenous mechanisms to inactivate the free radicals formed.

There is a second reason to propose that MDMA does not induce 5-HT neurotoxic damage in the human brain. All evidence suggests that human users have very severe or fatal acute adverse responses (primarily hyperthermia and its associated problems) to MDMA at plasma concentrations in excess of 1000 $ng{\cdot}mL^{-1}$. It is difficult to give a definite figure as there are remarkably few reports on severe adverse or fatal effects of the drug that also report drug exposure. However, several papers report severe problems and deaths with plasma concentrations in the range of 1000–1500 $ng{\cdot}mL^{-1}$ (Dowling *et al.*, 1987; Henry *et al.*, 1992). In contrast, monkeys and rats survive C_{max} in excess of 2000 $ng{\cdot}mL^{-1}$ (Mechan *et al.*, 2006; Green *et al.*, 2009 and see Figure 1). Indeed in the case of rats this degree of exposure has to be repeated several times in order to induce neurotoxicity. Humans are therefore unlikely to survive even a single acute dose of MDMA that produces plasma concentrations well below the exposure required to induce neurotoxic damage in rats or squirrel monkeys. By the same rationale 'binge-style' dosing by humans is also unlikely to increase the risk of neurotoxic damage because the inhibition of CYP2D6 induced by the first dose will increase the risk of acute adverse events, but also inhibit the metabolism of the drug to neurotoxic metabolites.

However, there are important caveats to this proposal. We are discussing the effect of MDMA administration alone and in controlled therapeutic conditions, not in hot crowded dance club conditions. Furthermore, and crucially, ingestion of other drugs (legal or illicit) may alter the metabolism of MDMA or the function of endogenous free radical trapping mechanisms and thereby increase the ability of MDMA to induce serotonergic damage.

Is MDMA producing other types of neurotoxic damage?

We are aware of the truism that it is impossible to prove a negative, so it is worth considering some limitations that affect our contention that MDMA does not cause neurotoxic damage to 5-HT neurones in the human brain. However, it is also worth emphasizing that MDMA is a drug and therefore obeys all the normal rules of pharmacology and an idiosyncratic response in any individual does not negate the accepted rules of dose–response relationships in either desired or adverse effects.

The limitation of most experimental work on MDMA-induced neurotoxicity is the reliability of the primary measure made as an index of intact 5-HT nerve terminals, namely loss of 5-HT content in the brain or brain region, or loss of other markers of 5-HT function such [^{3}H]-paroxetine binding. [^{3}H]-Paroxetine binding is probably a more accurate index of 5-HT neurotoxicity than amine or metabolite concentration as it is not susceptible to alterations in 5-HT synthesis or release (O'Shea *et al.*, 2006). Most of the animal reports on the extent of any 'neurotoxic' damage are assessed by post-mortem analysis of levels of 5-HT or [^{3}H]-paroxetine binding (although histological studies demonstrating neurological damage have been made; see Green *et al.*, 2003; Baumann and Rothman, 2009). Generally rather few forebrain regions have been studied and even these regions show varied sensitivity to the neurotoxic effect of MDMA (Sanchez *et al.*, 2004; O'Shea *et al.*, 2006; Rodsiri *et al.*, 2010). It is certainly not possible to discount the fact that a low dose of MDMA, although not producing damage in the usual major brain regions examined, is nevertheless inducing 5-HT terminal damage in some other discrete region or regions.

Several studies have observed long-term changes in behavioural response in rats following doses of MDMA that have not produced loss in 5-HT content or loss of [^{3}H]-paroxetine binding in any brain region examined. For example, Rodsiri *et al.* (2011) reported long-term disruption of novel object discrimination 2 weeks following 'binge-type' repeated low dose MDMA administration, but no loss of 5-HT in hippocampus, cortex or striatum. Similarly Fone *et al.* (2002) observed that MDMA administration to adolescent rats reduced social interaction and enhanced the subthreshold rewarding effect of cocaine at adulthood, despite an absence of 5-HT loss in cortex, hippocampus or brain stem or any alteration in [^{3}H]-paroxetine binding. The study of Bull *et al.* (2004) noted that MDMA pretreatment attenuated both the anxiogenic effect and the wet-dog shake behaviour elicited by the 5-HT_{2A} agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), suggesting that short-term exposure to MDMA may cause a long lasting alteration in 5-HT_{2A} receptor function. Again this change was not

accompanied by any alteration in [^{3}H]-paroxetine binding. One therefore has to be aware that 5-HT concentration or [^{3}H]-paroxetine binding may not be the only, or even the best, markers of neurotoxic-induced change. Long-term changes in function may be occurring, which are not directly due to 5-HT loss but could result from a long-term compensatory reinnervation or hyperinnervation of brain regions like the amygdala and hippocampus that regulate the response to environmental cues or to modifications in 5-HT receptor function such as a change in the $5HT_{2A}$ sensitivity as reported by Bull *et al*. (2004; 2006).

Conclusions

Nothing that has been reviewed in this paper should be taken to suggest that MDMA is always a safe recreational drug to ingest. What it does suggest is that most published studies on MDMA must be interpreted with caution when attempting to translate effects seen in animals to potential adverse effects in humans. Taking into account the differences in the pharmacokinetic profile of MDMA in rats and humans, what we also propose is that the doses currently being used to investigate the possible therapeutic benefits of the drug are unlikely to produce any severe acute or importantly any long-term neurotoxic damage in the human brain, particularly if given as a single acute administration in the absence of other agents in a therapeutic milieu.

Conflict of interest

The authors declare no conflict of interest.

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