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REVIEW

The preclinical pharmacology of mephedrone; not just MDMA by another name

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The substituted β-keto amphetamine mephedrone (4-methylmethcathinone) was banned in the UK in April 2010 but continues to be used recreationally in the UK and elsewhere. Users have compared its psychoactive effects to those of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy'). This review critically examines the preclinical data on mephedrone that have appeared over the last 2–3 years and, where relevant, compares the pharmacological effects of mephedrone in experimental animals with those obtained following MDMA administration. Both mephedrone and MDMA enhance locomotor activity and change rectal temperature in rodents. However, both of these responses are of short duration following mephedrone compared with MDMA probably because mephedrone has a short plasma half-life and rapid metabolism. Mephedrone appears to have no pharmacologically active metabolites, unlike MDMA. There is also little evidence that mephedrone induces a neurotoxic decrease in monoamine concentration in rat or mouse brain, again in contrast to MDMA. Mephedrone and MDMA both induce release of dopamine and 5-HT in the brain as shown by *in vivo* and *in vitro* studies. The effect on 5-HT release *in vivo* is more marked with mephedrone even though both drugs have similar affinity for the dopamine and 5-HT transporters *in vitro*. The profile of action of mephedrone on monoamine receptors and transporters suggests it could have a high abuse liability and several studies have found that mephedrone supports self-administration at a higher rate than MDMA. Overall, current data suggest that mephedrone not only differs from MDMA in its pharmacological profile, behavioural and neurotoxic effects, but also differs from other cathinones.

Abbreviations

5-HIAA, 5-hydroxyindoleacetic acid; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin; ACMD, Advisory Committee on the Misuse of Drugs; BRL44408, 2-((4,5-dihydro-1H-imidazol-2-yl)methyl)-2,3-dihydro-1-methyl-1H-isoindole; BSR, brain stimulation reward; C_{max}, peak plasma concentration; DAT, dopamine transporter; EF_{50}, half maximal response; EMCDDA, European Monitoring Centre for Drugs and Drug Addiction; MDMA, 3,4-methylenedioxymethamphetamine; PCPA, p-chlorophenylalanine; SCH23390, R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzapine; SERT, 5-HT transporter; t_{max}, time of drug peak

Introduction

Mephedrone (4-methylmethcathinone; Figure 1) was first synthesized in 1929 as a homologue of ephedrine, a year after the publication of the synthesis of another homologue, methcathinone (Figure 1). These two compounds have stimulant properties and methcathinone was actually marketed in the USSR as an antidepressant in the 1930s but became illegal in the USA and several other countries in the 1990s following evidence of widespread abuse (see Kelly, 2011). Bupropion, another chemically related compound, has been available in many countries since the mid 1980s, being initially marketed as an antidepressant, a treatment for attention deficit hyperactivity disorder and subsequently as an aid to smoking

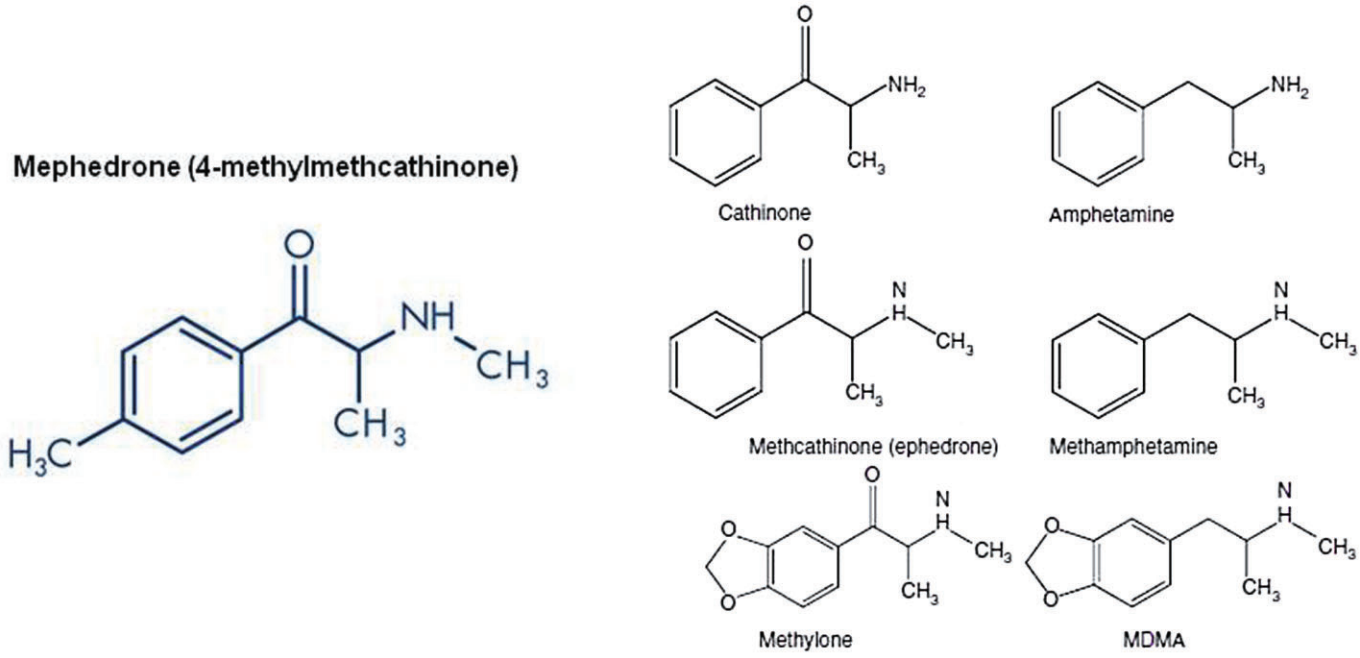

Figure 1
Structure of mephedrone and other β-keto amphetamines (cathinones) and their related amphetamine congeners.

cessation. Cathinone (Figure 1) itself is known to be the major active constituent of khat, the leaf from the *Catha edulis* plant that has been chewed recreationally in East Africa and parts of the Middle East for centuries. Khat is outlawed in the USA, Canada and many European countries, but remained legal in the UK. However, the government announced on 3 July 2013 that it intended to make this herbal stimulant a controlled class C drug like anabolic steroids and ketamine. For a more detailed historical overview on the history of the synthesis and clinical use of cathinone derivatives, see Kelly (2011).

Scientific interest in mephedrone was resurrected around the turn of the 21st century when the psychoactive effects of mephedrone were discovered and it became widely available as a party drug in Israel, selling under the nickname of 'plant food'. During this period, a number of other synthetic cathinones appeared as 'legal highs'. These so-called 'designer drugs' are compounds that are chemically related to known psychoactive substances but because of their novel structure have not been listed as controlled substances. 3,4-Methylenedioxymethcathinone (methylone; Figure 1), a cathinone with a close structural similarity to 3,4-methylenedioxymethamphetamine (MDMA), became available around 2004 in Japan and the Netherlands and its availability was enhanced by it being sold in head shops and on the Internet. Mephedrone with street names that include m-cat, drone, bubbles, bath salts and meow-meow (although the last has been suggested to have originated in the popular press) subsequently became available via these same sales outlets often being marketed as 'plant food' or 'bath salts'.

Because of the extensive recreational use of mephedrone it received substantial media attention in the UK, particularly as it was implicated in a number of adverse events and unexplained deaths and was banned in April 2010 following the advice of the Advisory Committee on the Misuse of Drugs (ACMD, 2010). The chair of the committee was reported to have stated that mephedrone 'is an amphetamine by another name' (Dyer, 2010). This was perhaps a surprising conclusion given that we have been unable to find in PubMed a single preclinical neuropharmacological publication on mephedrone before 2011 and very few on methcathinone. Mephedrone was also classified as a controlled substance in many other European countries in December 2010 (EMCDDA, 2011) despite the ACMD (2010) report opening with: 'There are no formal pharmacokinetic and pharmacodynamic studies on mephedrone. There are no published formal studies assessing the psychological or behavioural effects of mephedrone in humans. In addition, there are no animal studies on which to base an extrapolation of potential effects.' In the USA, President Barack Obama signed a Federal law banning mephedrone in July 2012 (Haggin, 2012). Interestingly, this law is similar to those enacted in Europe in that it covers many related cathinone substances.

Nevertheless, mephedrone continued (and continues) to be available for illicit recreational consumption (Brandt *et al.*, 2010). Before being banned, mephedrone was the cathinone derivative with the highest recreational use. An online survey of 2289 experienced polydrug users found that 42% had tried mephedrone at least once, with approximately 30% using it every 2 weeks or more frequently (Winstock *et al.*, 2011b). The increased use of mephedrone coincided with a decrease in both the availability and also in the purity of 'ecstasy' tablets. Fewer than 50% of ecstasy tablets confiscated in the Netherlands in 2009 contained MDMA, 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 MDMA/ecstasy. Both the decline in purity and availability of ecstasy tablets and the fact that mephedrone had initially been legal are thought to be the main reasons for its increased popularity (EMCDDA, 2010).

What was striking about the legislation that made mephedrone illegal was the way it was constructed. The original UK Misuse of Drugs Act (1971) only allowed a specific compound to be controlled. In contrast, not only mephedrone but also many other chemically related cathinone compounds were also banned with it, presumably in an attempt to outlaw the development and use of structurally related designer drugs. It was believed that this was the first time that a generic ban based purely on chemical structure had been enforced on a group of compounds (Morris, 2010). The advantages and problems of this type of generic approach to legislation have recently been reviewed (Van Amsterdam *et al.*, 2013).

What many may consider should be a matter of some concern is that the ban on mephedrone and related compounds appeared to have been driven more by information given in the media rather than peer-reviewed scientific knowledge gained from relevant clinical and preclinical pharmacological studies. Deaths and severe adverse reactions that were widely reported in the press as being the result of mephedrone ingestion were subsequently found to be due to other drugs or even natural causes (Measham *et al.*, 2010; Sare, 2011). Many of the newspaper reports on the effects of the drug in recreational users were hyperbolic, speculative or just incorrect. For example, one story in a major newspaper on a severe adverse event was actually an Internet hoax (Davey *et al.*, 2010). Needless to say, retraction of the false information seldom occurred. Even scientific papers on the adverse effects of the drug sometimes disclosed that evidence on the physiological and psychological consequences of ingesting the drug was based solely on the fact that the subjects under investigation 'believed' that they had taken mephedrone (Dargan *et al.*, 2010; James *et al.*, 2011; Regan *et al.*, 2011; Wood *et al.*, 2011). No forensic blood samples were taken to confirm that exposure to mephedrone had occurred. Interestingly, a recent study still found that a significant proportion of 'mephedrone fatalities' was likely to be due to the other drugs that had been subsequently identified *post-mortem* (Schifano *et al.*, 2012).

The last 2–3 years has seen the publication of a reasonable body of preclinical work on the pharmacology of mephedrone and this review is a critical appraisal of these studies. Clinical reviews on the drug are available elsewhere (Dargan *et al.*, 2010; 2011; Schifano *et al.*, 2011; Prosser and Nelson, 2012; Wood *et al.*, 2012; Wood and Dargan, 2012; Zawilska and Wojcieszak, 2013). Where relevant, we have compared the data on mephedrone with that obtained in studies on MDMA ('ecstasy'; Figure 1), because this is a substituted amphetamine and also because recreational users have subjectively reported that the stimulant, euphoric and empathogenic effects of mephedrone are similar to MDMA (Carhart-Harris *et al.*, 2011). Some users even consider mephedrone to be superior to MDMA in terms of the desired experience (Vardakou *et al.*, 2011; Winstock *et al.*, 2011b).

What is now becoming clear is that mephedrone has its own very specific pharmacology that is distinct from MDMA and also other amphetamines. As Dal Cason *et al.* (1997) concluded several years ago: 'caution [should] be used in attempting to draw conclusions or make predictions about the activity and potency of novel cathinone analogues by analogy to the structure–activity relationships derived from amphetamine-related agents; it would appear that each new cathinone analogue will require individual investigation.'

For simplicity, the information is grouped in subsections that examine its main pharmacokinetic and pharmacodynamic effects in experimental animals.

Metabolism and pharmacokinetics of mephedrone in rats and humans

Until recently, a major problem in assessing the pharmacological effects of MDMA was that few detailed pharmacokinetic studies had been performed in either animals or humans. Consequently, it was difficult to translate most of the preclinical pharmacodynamic studies of this drug in terms of their likely functional and toxicological importance. Recent pharmacokinetic studies have shown that MDMA has a much faster rate of metabolism in rats compared with humans (Baumann *et al.*, 2009). Consequently, many studies on both the pharmacological and toxicological effects of this drug in experimental animals have limited translational value and may even be misleading (Green *et al.*, 2012a). In contrast, there are already several good pharmacokinetic studies on mephedrone in rats (Hadlock *et al.*, 2011; Aarde *et al.*, 2013; Martínez-Clemente *et al.*, 2013; Miller *et al.*, 2013) and some useful results on the plasma concentrations of the drug in recreational users which allow a degree of confidence in the translational value of preclinical studies in rodents.

The normal routes of mephedrone administration in recreational users are reported to be oral and insufflation. Extrapolation from dosing to plasma levels is difficult as there are no detailed dose–concentration curves available and pharmacokinetic studies on the drug in humans have yet to be performed. However, it is suggested that a 'normal' recreational oral dose is 100–200 mg, while somewhat lower doses are used when the drug is insufflated (EROWID, 2013). This oral dose is similar to the usual oral MDMA dose typically resulting from ingestion of two tablets (140–180 mg), but an important difference with mephedrone is that the reported short duration of the psychoactive response often leads to rapid repeat dosing (Schifano *et al.*, 2012). Interestingly, plasma mephedrone concentrations in subjects suffering a fatal overdose have been reported to be in the region of 2000 $ng \cdot mL^{-1}$ (Maskell *et al.*, 2011; Schifano *et al.*, 2012) which is very similar in range to the concentration of MDMA seen *post-mortem* in persons suffering from fatal acute toxicity (Dowling *et al.*, 1987; Henry *et al.*, 1992). Therefore, extrapolation from MDMA recreational use would suggest that administration of mephedrone in the 5–10 $mg \cdot kg^{-1}$ range to rats is comparable to doses used recreationally and therefore appropriate when simulating a human recreational dose. However, this dose may not be sufficient to reflect the drug

exposure that must occur when humans engage in binge dosing. In mimicking that situation, repeat dosing of animals must also be performed.

In Sprague-Dawley rats, the uptake and elimination of a single dose of mephedrone (5.6 mg·kg^{-1} s.c.) is rapid. The peak plasma concentration (C_{max}) observed was 1206 ng·mL^{-1} with a t_{max} of 0.25 h (Miller *et al.*, 2013). In binge dosing studies, plasma levels of 384.2 ± 62.2, and 1294.3 ± 145.5 ng·mL^{-1} were recorded 1 h after, respectively, 4 × 10 or 4 × 25 mg·kg^{-1} s.c., given at 2 h intervals. Whole brain tissue levels of 2.1 ± 0.2 ng·mg^{-1} and 7.8 ± 0.9 ng·mg^{-1} were found 1 h after these dose schedules (Hadlock *et al.*, 2011). A further study in Sprague-Dawley rats given i.v. mephedrone (10 mg·kg^{-1}) reported plasma concentrations fitted a two-compartment model (α = 10.23 h^{-1}, β = 1.86 h^{-1}). The same study showed that after oral administration (30 and 60 mg·kg^{-1}), the peak mephedrone concentration occurred between 0.5 and 1 h later and the drug was undetectable at 9 h. The bioavailability of mephedrone was about 10% and the plasma protein binding value was 21% (Martínez-Clemente *et al.*, 2013).

The drug is rapidly taken up into the brain (peak levels were observed 2 min after i.v. injection) and almost totally cleared within an hour (Aarde *et al.*, 2013). Peak brain tissue concentrations were 4 ng·mg^{-1} 2 min after a 1 mg·kg^{-1} i.v. dose. The concentration had fallen to less than 1 ng·mg^{-1} within 30 min and less than 0.4 ng·mg^{-1} 60 min later (Aarde *et al.*, 2013). Consistent with this profile, Simmler *et al.* (2013) noted that in rats mephedrone had a twofold greater blood–brain barrier permeability than MDMA.

The Aarde *et al.* (2013) study found that mephedrone is cleared rapidly from both Sprague-Dawley and Wistar rats and the *in vitro* assay confirmed that the drug undergoes extensive hepatic metabolism. Martínez-Clemente *et al.* (2013) also concluded that the drug is subject to first pass metabolism. Pedersen *et al.* (2012) reported that cytochrome P450 2D6 is the main metabolic enzyme responsible for degradation of mephedrone in humans, the same enzyme that metabolizes MDMA (Tucker *et al.*, 1994).

A few investigations have examined the metabolic pathways of mephedrone in both rodents and man. Meyer *et al.* (2010) examined the metabolite pattern after oral administration of the drug to Wistar rats and the primary metabolites identified were nor-mephedrone, nor-dihydro-mephedrone, hydroxytolyl-mephedrone and nor-hydroxytolyl-mephedrone (Figure 2; Dybdal-Hargreaves *et al.*, 2013). Identification was primarily in plasma, but also in urine. Based on this information, the partly overlapping

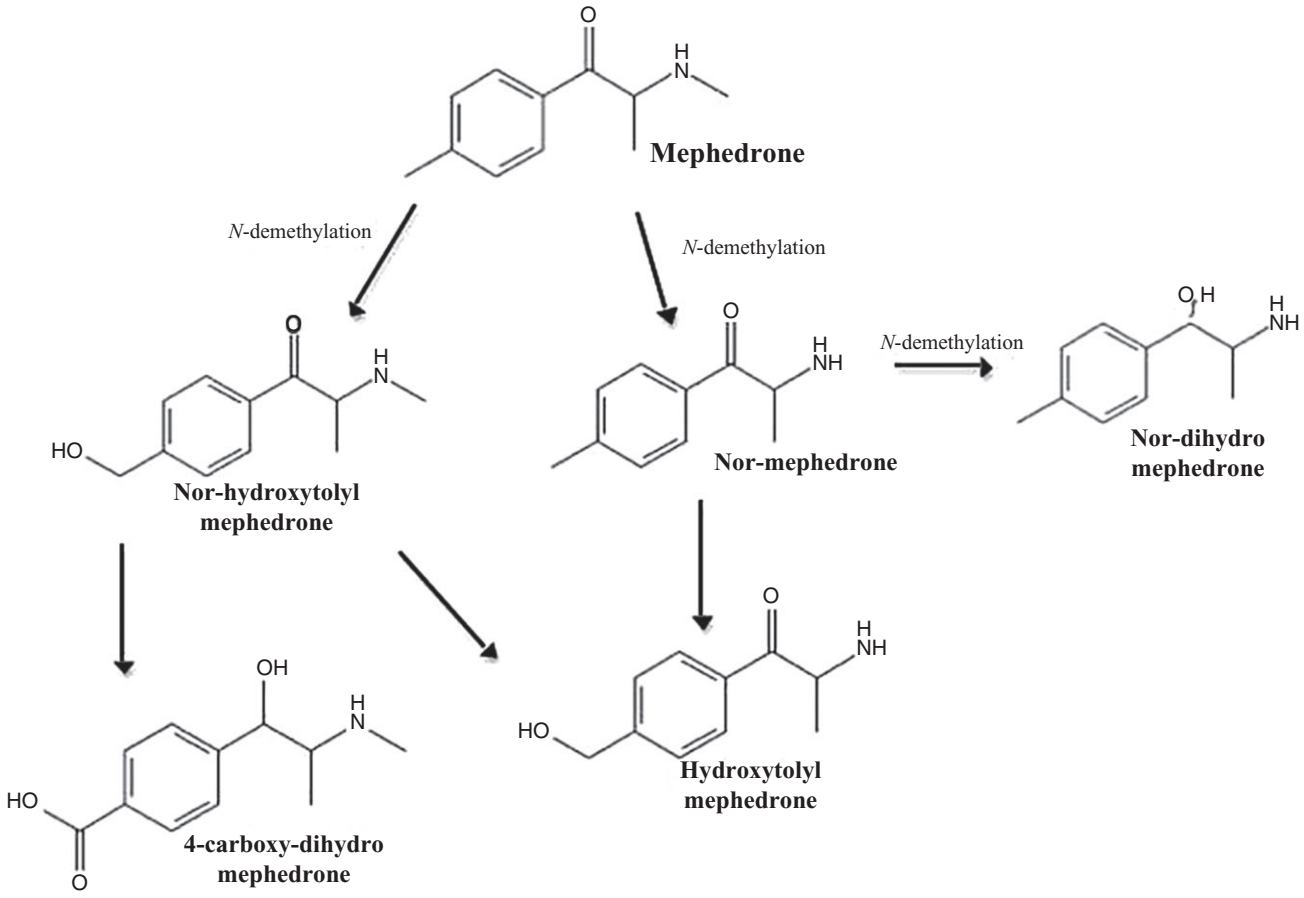

Figure 2

The major metabolites of mephedrone and proposed pathways of their formation. [Reproduced from Dybdal-Hargreaves *et al.* (2013) with permission from Elsevier Press].

metabolic pathways presented in Figure 2 have been postulated: N-demethylation to the primary amine, reduction of the keto moiety to the respective alcohol, and oxidation of the tolyl moiety to the corresponding alcohols. Because nor-hydroxytolyl-mephedrone and hydroxytolyl-mephedrone were more abundant after glucuronidase and sulphatase hydrolysis, it was concluded that they were partly excreted as glucuronides and/or sulphates. The same metabolites were identified in human plasma and urine but additionally 4-carboxy-dihydro-mephedrone was also identified in urine. These major metabolites were also detected by Martínez-Clemente *et al*. (2013) in their study on Sprague-Dawley rats. It is unclear at present whether any of the metabolites possess pharmacological activity. Further evidence for the formation of these metabolites both *in vitro* and *in vitro* has been presented by Pedersen *et al*. (2012), who used cDNA-expressed CYP enzymes and human liver microsomal preparations and found cytochrome CYP2D6 to be the main enzyme responsible for the *in vitro* metabolism of mephedrone, with some minor contribution from other NADPH-dependent enzymes. They also found both hydroxytolyl-mephedrone and nor-mephedrone were formed. In four forensic traffic accident cases where mephedrone was detected in blood, hydroxytolyl-mephedrone and nor-mephedrone, 4-carboxy-dihydro-mephedrone, dihydro-mephedrone, and 4-carboxy-mephedrone were all also detected.

MDMA is metabolized to catechol metabolites which can undergo oxidation to o-quinones that are highly redox-active molecules and produce free reactive oxygen species or nitrogen species radicals (Capela *et al*., 2006). It is widely believed that it is these oxidation products which may be responsible for the toxicity exerted by MDMA (Capela *et al*., 2009; Song *et al*., 2010; Green *et al*., 2012a), a view supported by the observation that administration of the free radical trapping agent α-phenyl-N-tert-butyl nitrone attenuated the long-term loss of 5-HT in the rat brain induced by MDMA (Colado and Green, 1995). In contrast to MDMA, catechol and quinone metabolites do not appear to be formed as the result of mephedrone metabolism (Figure 2). This distinction may explain why most studies have failed to observe any similar mephedrone-induced neurotoxicity in rat brain (see later).

The common practice of rapid binge dosing of mephedrone by recreational users (see Schifano *et al*., 2011; Winstock *et al*., 2011a) to sustain its psychoactive action is likely to reflect its rapid metabolism in humans. Furthermore, this is consistent with the proposal that the general pharmacokinetic profile of mephedrone is similar in rats and humans. This makes mephedrone markedly different from MDMA which has a rapid rate of metabolic clearance in rats and several other species but a much slower rate of metabolism in humans (Green *et al*., 2012a). MDMA also has a major metabolite 3,4-methylenedioxyamphetamine (MDA) which has the same general pharmacological activity as MDMA (Green *et al*., 2003; 2012a), while no active metabolites of mephedrone have yet been identified.

Locomotor activity

Increased locomotion following mephedrone administration has been observed in several strains of rats and mice. Kehr *et al*. (2011) noted that the locomotor effect of mephedrone in Sprague-Dawley rats was similar in intensity and duration to MDMA at the same dose (3 mg·kg^{-1} s.c.), but modest and short lasting compared with a lower dose of amphetamine (1 mg·kg^{-1} s.c.). The brief period of increased activity is consistent with the previously discussed short plasma half-life ($t_{½}$) of mephedrone, an interpretation supported by a recent study demonstrating a clear relationship between plasma mephedrone concentration and locomotor activity (Martínez-Clemente *et al*., 2013).

Lisek *et al*. (2012) also reported that mephedrone (3–30 mg·kg^{-1} i.p.) increased ambulatory activity in Sprague-Dawley rats and showed that locomotor hyperactivity was inhibited by pretreatment with the dopamine D_1 receptor antagonist SCH23390, but enhanced by pretreatment with sulpiride, a dopamine D_2 receptor antagonist (receptor nomenclature conforms to BJP's *Guide to Pharmacology*, Alexander *et al*., 2013). Shortall *et al*. (2013c) also observed a dose-dependent (1–10 mg·kg^{-1} i.p.) increase in locomotion in Lister Hooded rats lasting around 60 min after the highest dose. A subsequent study (S.E. Shortall *et al*., unpubl. obs.) using a greater dose range (4–30 mg·kg^{-1} i.p.) found that the highest dose enhanced further both the activity peak and AUC, but had only a small effect on the duration of the locomotor response (Figure 3). Oral administration also induces a dose-dependent increase in locomotion but with a more sustained duration of action of around 2 h (Martínez-Clemente *et al*., 2013).

Wright *et al*. (2012a) examined the locomotor response in both Wistar and Sprague-Dawley strains in two different ambient temperature conditions (23 and 27°C). Although mephedrone increased locomotor activity for a similar duration in both strains, significantly more activity was observed in Sprague-Dawley rats. The locomotor activity was similar when the rats were examined in either ambient temperature condition, in contrast to the effect of mephedrone on body temperature (see later). However, in Sprague-Dawley rats, a

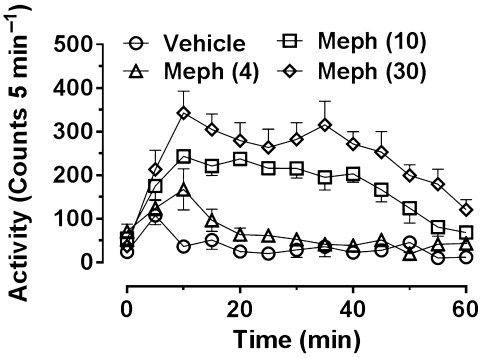

Figure 3

Locomotor response of individually housed male Lister-hooded rats following various doses of (±)-mephedrone HCl (4, 10 and 30 mg·kg^{-1} i.p.) administered at time 0. Rats were habituated to the test arena for 60 min prior to injection. Data are shown as mean ± SEM infrared beam breaks in each 5 min bin. Mephedrone-treated groups different from control group ($P < 0.01$ or better) as follows: mephedrone (4 mg·kg^{-1}) at 10 min; mephedrone (10 mg·kg^{-1}) from 10 to 45 min; and mephedrone (30 mg·kg^{-1}) from 10 to 55 min.

higher ambient temperature of 30°C has been reported to enhance mephedrone-induced locomotion compared with that seen at 20°C (Miller *et al*., 2013).

Motbey *et al*. (2012a) administered the high dose of 30 mg·kg^{-1} i.p and noted a marked hyperlocomotion response over the next 60 min in Wistar rats which was not sensitized (enhanced in amplitude) even after this dose had been given once daily for 10 days. However, they also reported that no sensitization occurred in a parallel cohort given methamphetamine using the same dosing schedule. Because methamphetamine, amphetamine and MDMA are all known to produce sensitization when given at a low dose and with an abstinence period (Vanderschuren and Kalivas, 2000; Aberg *et al*., 2007; Bradbury *et al*., 2012), this suggests that the protocol employed may not have been appropriate for investigating sensitization, particularly as sensitization to the locomotor effects of mephedrone has been reported by several other groups as detailed below. Lisek *et al*. (2012) gave mephedrone (0.5 mg·kg^{-1} i.p.) once daily for 5 days followed by a 10 day abstinence period while Shortall *et al*. (2013c) gave 10 mg·kg^{-1} i.p. on 2 consecutive days each week for 3 weeks in order to mimic likely weekend recreational dosing in humans. Both groups observed robust enhancement of the locomotor response on the final test day compared with that seen following the first injection. In a further study, Shortall *et al*. (unpublished) gave a total of 5 doses of either mephedrone (10 mg·kg^{-1} i.p.) or MDMA (5 mg·kg^{-1} i.p.) to reflect weekend human use (2 consecutive days in weeks 1 and 2, and a final dose after a further week) and saw a robust enhancement (sensitization) of the locomotor response to both compounds following the final versus the first dose (Figure 4). Gregg *et al*. (2013) have also recently reported that two different dose schedules (both fixed and variable dose schedules) to Sprague-Dawley rats produced clear evidence of locomotor sensitization.

Huang *et al*. (2012) compared the locomotor stimulant effects of mephedrone (1–10 mg·kg^{-1} s.c.), d-methamphetamine (0.5–5.6 mg·kg^{-1} s.c.) and MDMA (1–7.5 mg·kg^{-1} s.c.) on voluntary wheel running activity in Wistar rats. Methamphetamine induced a biphasic pattern of counts with relatively higher activity following lower doses, and lower counts following the highest dose, probably due to the induction of stereotyped behaviour at the high dose (Huang *et al*., 2012). In contrast, both mephedrone and MDMA, neither of which has been reported to induce stereotypic behaviour, produced a monophasic, dose-dependent reduction in counts compared with saline-treated controls. Although such a decrease seems paradoxical when compared with the consistent increase in locomotion recorded in activity boxes, this is because spontaneous wheel running represents a different form of activity involving divergent behavioural processes from spontaneous activity.

Following mephedrone, Baumann *et al*. (2011) observed reciprocal forepaw treading, which is one component of the 5-HT syndrome in rats (Green and Grahame-Smith, 1976). Components of this syndrome also occur following MDMA, and although the response is more robust, with the expression of other components of the syndrome, it is only apparent after a high dose (Colado *et al*., 1993). The fact that MAO is also inhibited by MDMA (Leonardi and Azmitia, 1994) may assist in the production of the syndrome. In the absence of a

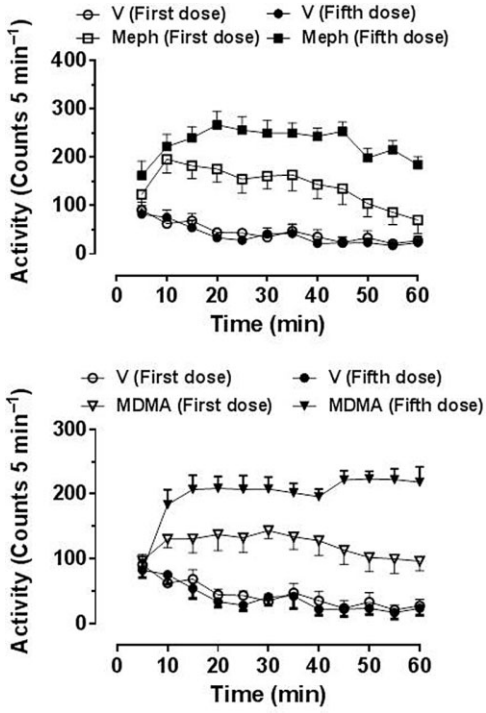

Figure 4

Locomotor response of individually housed rats following the first and fifth doses of (±)-mephedrone HCl (10 mg·kg^{-1}) or (±)-MDMA HCl (5 mg·kg^{-1}), doses being given on 2 consecutive days on weeks 1 and 2, and the final dose 1 further week later. Rats were habituated to the test arena for 60 min prior to injection. Data are shown as mean ± SEM infrared beam breaks in each 5 min bin. Total beam breaks are first dose: saline 534 ± 74, mephedrone 1695 ± 300*, MDMA 1447 ± 181*; fifth dose: saline 467 ± 74, mephedrone 2742 ± 213*†, MDMA 2629 ± 319*†. *Compared with the respective dose of the saline injection; $^{\dagger}P < 0.001$ compared with the first dose of the same drug challenge injection.

MAO inhibitor or a 5-HT re-uptake inhibitor, it is difficult to induce the syndrome in rats (Green and Grahame-Smith, 1976). Interestingly, there is one clinical case report of the 5-HT syndrome in a mephedrone user, but the patient was also taking fluoxetine so it is likely that it is the combination that was responsible (Garrett and Sweeney, 2010). It has also been reported that 'bath salts' can induce the syndrome in recreational users (Joksovic *et al*., 2012; Rasimas, 2013).

Mephedrone administration also increases locomotor activity in mice. Both López-Arnau *et al*. (2012) and Marusich *et al*. (2012) observed a dose-dependent increase in locomotion without any accompanying increase in rearing *behaviour* (López-Arnau *et al*., 2012). Pretreatment with the 5-HT$_2$ receptor antagonist ketanserin or the non-selective dopamine receptor antagonist haloperidol, given at doses that did not affect basal locomotor activity, partly inhibited mephedrone-induced hyperactivity (by about 53 and 65% respectively). Pretreatment with p-chlorophenylalanine (PCPA), an inhibitor of 5-HT synthesis, also reduced the hyperlocomotor effect of mephedrone. In contrast, PCPA does not alter MDMA-induced hyperactivity in the mouse (Fantegrossi *et al*., 2005)

and the role of dopamine is also unclear. Benturquia *et al.* (2008) reported that the selective D_1 receptor antagonist SCH23390 antagonized the MDMA-induced locomotor response, but Risbrough *et al.* (2006) demonstrated that D_1 receptor activation inhibited straight-line activity. They also noted that D_2 receptor activation appeared to contribute to the repetitive circling behaviour produced by MDMA.

Body temperature and cardiovascular function

Hyperthermia is a major acute adverse event that can follow ingestion of MDMA by recreational users (Green *et al.*, 2003; Docherty and Green, 2010; Halpern *et al.*, 2011; Parrott, 2012a), and is sometimes marked in young persons who have ingested the drug at dance clubs or parties where the ambient temperature is high. These individuals sometimes also present with problems associated with hyperthermia, including rhabdomyolysis, myoglobinuria, renal failure, liver damage and disseminated intravascular coagulopathy, which can be fatal. Although administration of high or repeated doses of MDMA to rats usually causes hyperthermia, it can produce hypothermia particularly following a low dose or when the animals are housed singly or in a cool ambient temperature (Docherty and Green, 2010). Nevertheless, both MDMA-induced hyper- and hypothermia result from monoamine release in the brain (Docherty and Green, 2010).

Cathinone has been reported to induce hyperthermia and thermogenesis in urethane-anaesthetized rats (Tariq *et al.*, 1989), and hyperthermia in freely moving animals (Shortall *et al.*, 2013a). It also induces hyperthermia in the Siberian hamster (Jones *et al.*, 2014). Methcathinone also produces hyperthermia in both individually restrained (Rockhold *et al.*, 1997) and freely moving rats (Shortall *et al.*, 2013a). These reports confirm that cathinones have effects on temperature regulation at similar doses to those that affect locomotor behaviour. Furthermore, several reports indicate that recreational users of mephedrone can suffer from apparent changes in body temperature with cold or blue fingers commonly featuring among the recorded adverse events (ACMD, 2010; Schifano *et al.*, 2011; Winstock *et al.*, 2011a). Although incidences of hot flushes and sweating are sometimes reported (Schifano *et al.*, 2011) with mephedrone, severe hyperthermia has not been recorded (Wood *et al.*, 2010; 2011; Dargan *et al.*, 2011). Consequently, these indications that mephedrone might be altering thermoregulation in humans, coupled with earlier reports that both cathinone and methcathinone administration produces hyperthermia in rodents, spurred several groups to examine in detail the effect of mephedrone on body temperature and thermoregulation in rats.

At normal ambient room temperature (20°C), mephedrone, like MDMA, produces a hypothermic response in individually housed rats, although the mephedrone effect was transient (Figure 5; Shortall *et al.*, 2013a). Miller *et al.* (2013) also observed a body temperature decrease following mephedrone when the rats were housed at 20°C, but this decrease was abolished by housing at 30°C. Yet hyperthermia did not occur as would be expected with MDMA. Group housing also

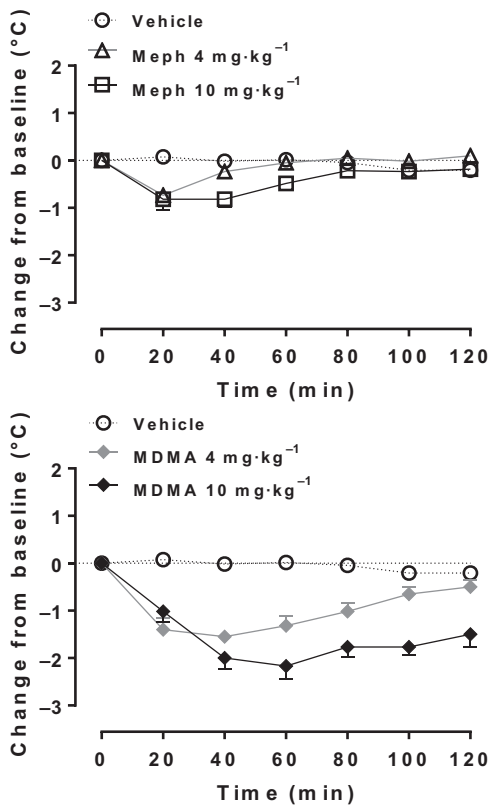

Figure 5

Effect of MDMA and mephedrone on rectal temperature in individually housed male Lister-hooded rats ($n = 5$–6 per group). Compounds (4 or 10 mg·kg^{-1} HCl salt) or saline vehicle (1 mL·kg^{-1}) were injected i.p. at 0 min and temperature assessed at 20 min intervals for the next 2 h. Data are shown as change in temperature (°C, mean ± SEM) from the baseline reading taken at the time of injection. [Reproduced from data presented in Shortall *et al.* (2013a)].

abolished the hypothermic response to mephedrone in rats and failed to induce hyperthermia (Shortall *et al.*, 2013a) as would have occurred following MDMA (Green *et al.*, 2003; Docherty and Green, 2010).

Two recent studies found that repeated dosing of mephedrone on the same day (binge dosing) induced hyperthermia. One study examined individually housed rats at normal ambient temperatures (three doses of 3–10 mg·kg^{-1} s.c.; Baumann *et al.*, 2011) while the other investigated group-housed rats in a warm (≥27°C) environment (four doses of 1–25 mg·kg^{-1} s.c.; Hadlock *et al.*, 2011). In contrast, in our own recent study on repeated dosing in individually housed rats, we again observed hypothermia (S.E. Shortall *et al.*, unpubl. obs.). The reason for the discrepancy between these findings is unclear but may relate either to dosing [the studies of Baumann *et al.* (2011) and Hadlock *et al.* (2011) both employed high cumulative dosing] or to strain differences. Wright *et al.* (2012a) found mephedrone-induced hypothermia in Wistar rats housed at 23 or 27°C but that even the highest dose given (10 mg·kg^{-1}) produced little body temperature change in Sprague-Dawley rats. Because both strains responded with a similar hypothermic response to the 5-

HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), this suggests that there is a strain-specific difference in the body temperature response to mephedrone rather than some generalized difference in their ability to respond to temperature-changing drugs.

Shortall *et al*. (2013a) reported several differences between the pharmacology of MDMA- and mephedrone-induced changes in body temperature. The mephedrone-induced hypothermia was prolonged by the α_1-adrenoceptor antagonist prazosin, but unaffected by the α_{2A}-adrenoceptor antagonist BRL 44408 that potentiates and prolongs MDMA-induced hypothermia (Bexis and Docherty, 2006). Mephedrone-induced hypothermia was unaffected by dopamine D_2 receptor blockade, which prevents MDMA-induced hypothermia (Green *et al*., 2005). However, it was prolonged and potentiated by the dopamine D_1 receptor antagonist SCH23390, which fails to affect MDMA-induced hypothermia in a cool environment (Green *et al*., 2005). These pharmacological differences are surprising given that mephedrone and MDMA share similar affinities for the human α_{2A}-adrenoceptor and possibly also the human D_1 and D_2 receptors (Simmler *et al*., 2013).

In an attempt to further understand the mechanisms involved in the induction of hypothermia following mephedrone, several groups have investigated its effect on cardiovascular function. Shortall *et al*. (2013a) compared the effect of MDMA and mephedrone on tail temperature because this is a major heat loss organ in the rat (Redfern *et al*., 1995) and also provides an indication of peripheral vascular tone. MDMA administered to singly housed rats at normal ambient temperature decreased tail temperature, indicative of peripheral vasoconstriction. This would aid heat conservation and may be mediated via a direct effect on α_{2A}-adrenoceptors (Bexis and Docherty, 2006; Simmler *et al*., 2013) that regulate tail blood flow and heat loss in the rat. However, the fact that the MDMA-induced decrease in tail temperature was both short in duration and modest in size compared with the concomitant long-lasting and major decrease in rectal temperature demonstrates that centrally regulated heat conservation mechanisms are disrupted after a single dose of MDMA (Green *et al*., 2005), as discussed in detail elsewhere (Docherty and Green, 2010). Under these same conditions, mephedrone also produced hypothermia, but the small and short-lasting decrease in rectal temperature was associated with a prolonged decrease in tail temperature, and therefore differed from the temporal profile of MDMA-induced thermoregulatory response. The decrease in tail temperature following mephedrone is consistent with its affinity for both α_1- and α_{2A}-adrenoceptors (Simmler *et al*., 2013), the recently reported hypertension produced in rats (Meng *et al*., 2012) and the side effects of cold or blue fingers experienced by recreational users (ACMD, 2010; Schifano *et al*., 2011; Winstock *et al*., 2011a).

Mephedrone, like MDMA (Hysek *et al*., 2012a,b,c), increases plasma noradrenaline levels (Shortall *et al*., 2013a), and in the case of mephedrone this effect is sensitive to α_1-adrenoceptor, α_{2A}-adrenoceptor and dopamine D_1 receptor blockade.

Further evidence for an action of mephedrone on peripheral adrenergic mechanisms has been obtained by two investigations on cardiovascular function in the rat. Varner *et al*. (2013) showed that mephedrone elicited dose-related increases in arterial pressure lasting around 1.5 h. This pressor response was accompanied by tachycardia that reached a plateau after 1 $mg \cdot kg^{-1}$ i.v. 2–5 min after drug administration. Similarly, Meng *et al*. (2012) reported that mephedrone (3 or 15 $mg \cdot kg^{-1}$ s.c.) significantly increased arterial pressure and heart rate in the conscious rat. The responses following s.c. injection had a slower onset and much longer duration (up to 5 h) than those following i.v. injection. The pressor response was significantly attenuated by phentolamine, indicating that activation of peripheral α-adrenoceptors plays an important role in mediating mean arterial pressure (MAP) responses. In contrast, the tachycardia produced by mephedrone was blocked by atenolol, suggesting that it was elicited by β-adrenoceptor activation. The authors proposed that mephedrone might release noradrenaline from peripheral sympathetic nerves innervating the vasculature; this is supported by the observation that mephedrone increases plasma noradrenaline (Shortall *et al*., 2013a). However, because Meng *et al*. (2012) showed that mephedrone elicits pressor responses and tachycardia in reserpine-treated rats, this suggests that the cardiovascular responses may not directly result from the release of noradrenaline from peripheral sympathetic nerves. Varner *et al*. (2013) therefore proposed that mephedrone has a substrate activity at noradrenaline transporters (NET) resulting in the release of cytoplasmic stores of noradrenaline, an idea supported by evidence of the affinity of mephedrone for these transporters (see later). All these observations in animals are consistent with the clinical reports that mephedrone can produce hypertension and tachycardia in humans (Wood *et al*., 2010; Regan *et al*., 2011).

Meng *et al*. (2012) extended their observations to examine the effects of mephedrone at a high concentration (30 μM) on cardiac electrophysiology and found little or no effect on the cardiac action potential waveform or L-type Ca^{2+} channels using ventricular myocytes isolated from the guinea pig, or on the transfected human ether-a-go-go-66 related gene cardiac K^+ channel. They also examined the action of mephedrone on cardiac function in rats in real time using echocardiography. Mephedrone produced dose-dependent effects on the heart that were consistent with sympathomimetic stimulation. A dose of 10 $mg \cdot kg^{-1}$ s.c. produced a clear increase in heart rate, stroke volume and cardiac output. Ejection fraction and fractional shortening, both indicators of cardiac contractility, were also significantly increased. The effects of mephedrone on the heart after i.v. injection were rapid but also short lived, with many of the parameters returning to near pre-dose values 10 min after administration.

MDMA also increases heart rate, MAP and cardiac output in both rats and humans (Lester *et al*., 2000; O'Cain *et al*., 2000; Pedersen and Blessing, 2001; Badon *et al*., 2002; Cole and Sumnall, 2003). In the rat-isolated right ventricle, MDMA potentiated contractions mediated by noradrenaline, but not those mediated by isoprenaline, consistent with an action at the NET (Al-Sahli *et al*., 2001). These results suggest that any cardiac stimulant actions of MDMA and mephedrone may involve indirect sympathomimetic effects. It is also possible that an increase in locomotor activity may increase heart rate,

but this is unlikely in human studies on MDMA where low doses were given in controlled clinical conditions.

Effect on brain monoamine concentrations

Only two studies have been published on the effect of mephedrone on brain tissue monoamine concentrations measured shortly after drug administration. Motbey *et al.* (2012a) examined concentrations of dopamine, 5-HT, and their major metabolites in striatum and hippocampus 60 min after either a single 30 mg·kg^{-1} dose or 60 min after the final of 10 once daily injections of this dose. Dopamine was significantly elevated, while 5-HT was significantly decreased, in the striatum and hippocampus following a single dose. A similar pattern was seen 60 min after 10 once daily injections. These data have recently been supported by Shortall and colleagues (unpublished) who gave mephedrone (10 mg·kg^{-1} i.p.) on 2 consecutive days a week for 3 weeks and also observed reduced hippocampal 5-HT levels 60 min after the final dose.

These results are similar to the acute effect of MDMA on cerebral monoamine content. Colado and Green (1994) showed that MDMA produced a rapid decrease in 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) content and increased striatal dopamine. While Motbey *et al.* (2012a) observed a modest increase in 5-HIAA following mephedrone, Shortall *et al.* (unpublished) observed a decrease in 5-HIAA in both the hippocampus and the striatum, and also found that mephedrone and MDMA produced a similar decrease in striatal 3,4-dihydroxyphenylacetic acid (Shortall *et al.*, 2013c). Any discrepancies in the levels of monoamine or metabolites between studies are almost certainly due to rapid changes in their synthesis, release, metabolism and clearance shortly after drug-administration. Although metabolite concentrations cannot be used to indicate turnover rates as suggested by Motbey *et al.* (2012a), because neither 5-HT nor dopamine metabolism is at steady state (see Neff *et al.*, 1969; Costa *et al.*, 1972), we concur that their data suggest mephedrone releases dopamine and 5-HT from nerve terminals, results also strongly supported by several microdialysis studies.

By implanting microdialysis probes in the nucleus accumbens, Kehr *et al.* (2011) found that mephedrone (3 mg·kg^{-1} s.c.) rapidly increased extracellular dopamine levels in conscious rats by almost 500% above basal, while the same dose of MDMA induced a more modest increase (235%). The increase in extracellular 5-HT was 941% following mephedrone and 911% after MDMA. Baumann *et al.* (2011) obtained very similar results in the same brain region, showing that 0.3 and 1.0 mg·kg^{-1} of mephedrone i.v. produced a dose-related increase in extracellular dopamine and 5-HT, with the magnitude of the effect on 5-HT being greater. The microdialysis studies of Wright *et al.* (2012a) also observed that mephedrone induced a larger increase in 5-HT compared with dopamine in the nucleus accumbens. These data present an interesting contrast to the effect of MDMA on monoamine release in this region, because this drug produces twofold greater release of dopamine than of 5-HT (O'Shea *et al.*, 2005). Recently, we have found that mephedrone also increases the extracellular concentration of dopamine in the striatum (S.E. Shortall *et al.*, unpubl. obs.).

Neurotoxicity

It is well established that high-dose amphetamine or methamphetamine administration to rats or mice induces neurotoxic damage to both dopamine and 5-HT nerve endings in the brain (Hotchkiss and Gibb, 1980; Armstrong and Noguchi, 2004; Cadet *et al.*, 2007). In contrast, repeated MDMA administration to the rat, guinea pig and monkey induces a selective neurotoxic loss of 5-HT in forebrain regions (see Green *et al.*, 2003). The severity of this loss is dependent both on dose and frequency of administration (O'Shea *et al.*, 1998) and the ambient temperature at the time of drug administration. By contrast, MDMA fails to produce damage to 5-HT neurons in mouse brain, instead causing damage to dopamine nerve terminals (see Green *et al.*, 2003). However, the different pharmacokinetics of MDMA in humans compared with rats makes it unlikely that this compound is neurotoxic in human brain (Green *et al.*, 2012a). Nevertheless, neurotoxicity may occur in humans when it has been taken in conjunction with other drugs, as generally occurs (Green *et al.*, 2012b; Parrott, 2012b).

This association of amphetamines with neurotoxic damage to amine nerve terminals in the brain encouraged several groups to examine whether mephedrone also induced neurotoxicity in the rodent brain. The first study used an intense dosing regimen (four doses of 10 or 25 mg·kg^{-1} s.c. at 2 h intervals) and reported neurotoxic loss of 5-HT in the hippocampus of Sprague-Dawley rats (Hadlock *et al.*, 2011). The neurotoxic damage reported by Hadlock *et al.* (2011) also produced a loss in the 5-HT transporter (SERT), analogous to the situation with MDMA (O'Shea *et al.*, 2006).

However, several subsequent investigations have failed to confirm the Hadlock *et al.* (2011) finding that mephedrone produces neurotoxic loss of 5-HT in the rat brain. A similar binge-type dosing schedule of three injections of mephedrone (3 or 10 mg·kg^{-1} s.c.), one being given every 2 h, produced no loss of 5-HT, dopamine or noradrenaline in the cortex or striatum 2 weeks after dosing (Baumann *et al.*, 2011). Similarly, administration of mephedrone (10 mg·kg^{-1}) on 2 consecutive days a week for 3 weeks (to mimic weekend-type recreational use in humans) also failed to alter tissue concentrations of dopamine or 5-HT in the hippocampus, striatum or frontal cortex (Shortall *et al.*, 2013c). A dose schedule of 7.5, 15 or 30 mg·kg^{-1} of mephedrone once daily for 10 days also failed to produce any long-term loss of 5-HT or dopamine in the striatum or hippocampus (Motbey *et al.*, 2012a). Another binge dosing study in rats giving mephedrone (30 mg·kg^{-1} twice daily for 4 days) also failed to produce any loss of 5-HT, dopamine, noradrenaline or their metabolites, or noradrenaline in the frontal cortex, hippocampus or striatum 2 weeks later (den Hollander *et al.*, 2013). This study also found that 5-HT and dopamine transporter levels were unchanged in these *in vivo* studies (den Hollander *et al.*, 2013).

Baumann *et al.* (2013a) suggested that a possible reason for apparent neurotoxicity reported by Hadlock *et al.* (2011) might be group housing of the rats, but this seems unlikely as

den Hollander *et al*. (2013) also grouped their animals. Furthermore, Motbey *et al*. (2012a) and den Hollander *et al*. (2013) gave high repeated doses that would have been more than sufficient to cause toxicity if it had been MDMA that had been administered at the same dose (O'Shea *et al*., 1998).

Studies in mice by Angoa-Pérez *et al*. (2012) and den Hollander *et al*. (2013) failed to detect any loss in striatal dopamine terminal integrity, as indicated by measuring dopamine levels, tyrosine hydroxylase activity and dopamine transporter (DAT) protein levels 7 days after administering a total of four doses of mephedrone (40 mg·kg^{-1}) at 2 h intervals (Angoa-Pérez *et al*., 2012) or 30 mg·kg^{-1} twice daily for 4 days (den Hollander *et al*., 2013). Furthermore, mephedrone did not cause microglial activation in the striatum or increase glial fibrillary acidic protein (GFAP) levels, both reliable markers of neurodegeneration (Angoa-Pérez *et al*., 2012; den Hollander *et al*., 2013). This contrasts strongly with MDMA where a similar dose regime results in a substantial loss of striatal dopamine (Logan *et al*., 1988; O'Shea *et al*., 2001) and an increase in GFAP (Miller and O'Callaghan, 1995; Johnson *et al*., 2002a,b).

In a subsequent study, Angoa-Pérez *et al*. (2013) administered mephedrone (10, 20 or 40 mg·kg^{-1}) to mice before each injection of methamphetamine (four injections of 2.5 or 5.0 mg·kg^{-1} at 2 h intervals). This methamphetamine dosing regime produced the expected dopamine neurotoxicity in the striatum, decreasing dopamine, DAT and tyrosine hydroxylase levels. Mephedrone failed to produce any neurotoxic damage, but enhanced the methamphetamine-induced dopamine neurotoxicity. Mephedrone also enhanced the neurotoxic effects of amphetamine and MDMA on dopamine nerve endings, suggesting that a potentially dangerous interaction might occur if mephedrone is taken either intentionally or unintentionally with other illicit amphetamines.

Effect on monoamine receptors and transporters

There is now good evidence that mephedrone interacts with plasma membrane transporters, including the DAT, NET and 5-HT (SERT) (Baumann *et al*., 2011; Hadlock *et al*., 2011; López-Arnau *et al*., 2012; Martínez-Clemente *et al*., 2012; Simmler *et al*., 2013). As Baumann *et al*. (2013a) emphasized, drugs acting on these transporters can be classified as either substrates (e.g. amphetamine) or blockers (e.g. cocaine). Substrates (but not blockers) are transported into the cell where they disrupt vesicular storage and stimulate non-exocytotic monoamine release by reversing the transporter flux (Rothman and Baumann, 2003; Sitte and Freissmuth, 2010), and may also interact with vesicular monoamine transporters. Blockers, in contrast, produce sustained deficits including monoamine depletion and loss of transporter function (Baumann *et al*., 2007; Fleckenstein *et al*., 2007). Several groups have reported that mephedrone inhibits the uptake of [^{3}H]-dopamine, [^{3}H]-noradrenaline and [^{3}H]-5-HT into brain tissue, suggesting it functions as a transporter blocker (Baumann *et al*., 2011; 2013a; Hadlock *et al*., 2011; López-Arnau *et al*., 2012; Martínez-Clemente *et al*., 2012; Simmler *et al*., 2013). However, as Baumann *et al*. (2013a) point out, assays measuring inhibition of uptake do not discriminate between drugs acting as transporter substrates or as blockers. Using *in vitro* release assays in rat brain synaptosomes (Rothman *et al*., 2001; Rothman and Baumann, 2003; Nagai *et al*., 2007), mephedrone was found to be a substrate for monoamine transporters, stimulating the release of [^{3}H]-1-methyl-4-phenylpyridinium ([^{3}H]-MPP+) via DAT and NET and release of [^{3}H]-5-HT via SERT (Baumann *et al*., 2011). Their results showed that mephedrone and MDMA cause non-selective release of monoamines by being substrates for all the transporters, while amphetamine is a selective substrate at both DAT and NET. Furthermore, mephedrone and MDMA both had a similar potency to each other as a releaser at all three monoamine transporters. The work of Baumann *et al*. (2011; 2013a) and Eshleman *et al*. (2013) on mephedrone and other cathinones makes it clear that their pharmacology can differ both from each other and from MDMA and amphetamine.

Another substantial study on the effect of a range of cathinones and MDMA is that of Simmler *et al*. (2013) who used transfected cells expressing human DAT, NET and SERT as their assay system. Again, their results pointed to mephedrone functioning as a transportable substrate. The potency of inhibition (IC_{50}) of mephedrone at DAT and SERT was similar, in agreement with the earlier studies of Hadlock *et al*. (2011) obtained using rat synaptosomes. MDMA was approximately 10 times more effective at the SERT than the DAT in Simmler *et al*.'s (2013) study and four times more in the synaptosomal study of Hadlock *et al*. (2011).

Mephedrone also binds to the 5-HT_{2A} receptor with low micromolar affinity (López-Arnau *et al*., 2012; Martínez-Clemente *et al*., 2012; Simmler *et al*., 2013) while MDMA has a slightly lower affinity (Simmler *et al*., 2013). Mephedrone has little affinity for the 5-HT_{1A}, 5-HT_{2C} or any dopamine receptor subtypes, in common with MDMA (Simmler *et al*., 2013). However, mephedrone (Simmler *et al*., 2013) and MDMA (Bexis and Docherty, 2006) both bind to the α_{2A}-adrenoceptor in the 1–10 μM range which may explain the peripheral vasoconstriction produced by both drugs as discussed earlier.

In a study to determine rat brain areas activated by mephedrone administration, Motbey *et al*. (2012b) examined the effects of mephedrone (15 and 30 mg·kg^{-1} i.p.) and methamphetamine (2 mg·kg^{-1} i.p.) on the expression of the c-fos transcription factor, an established marker of neuronal activation (Kovacs, 2008). The pattern of Fos expression induced by mephedrone resembled those expected for a drug combining the properties of methamphetamine and MDMA, with particularly strong Fos expression in the cortex, dorsal and ventral striatum, ventral tegmental area (typical of both MDMA and methamphetamine) and supraoptic nucleus (typical of MDMA), as demonstrated in earlier studies with methamphetamine (Carson *et al*., 2010) and MDMA (Hargreaves *et al*., 2007).

Effects in behavioural tests

Repeated use of MDMA by humans can lead to cognitive deficits (Parrott, 2013). However, meta-analysis does not

suggest a clear dose-related association but implies that a combination of MDMA with other recreational drugs may be more problematic (Verbaten, 2003; Laws and Kokkalis, 2007). Preclinical studies indicated that MDMA can impair working memory (Piper and Meyer, 2004; Rodsiri *et al.*, 2011) and sensorimotor gating in rats (Vollenweider *et al.*, 1999) without any concomitant neurotoxic damage (Rodsiri *et al.*, 2011). Therefore, Shortall *et al.* (2013c) compared the effect of mephedrone and MDMA on these measures. Mephedrone (1, 4 or 10 mg·kg^{-1}) or MDMA (10 mg·kg^{-1}) was injected on 2 consecutive days a week for 3 weeks (to mimic weekend-type recreational use in humans), and novel object discrimination (NOD; day 2), conditioned emotional response (CER; days 8 and 9) and prepulse inhibition of the acoustic startle (PPI; day 15) were evaluated. Rats that had received two previous treatments with mephedrone or MDMA (at 24 h and 30 min before testing) were unable to distinguish between the novel and familiar object during the choice trial, in contrast to controls. However, during the familiarization trial, mephedrone (4 and 10 mg·kg^{-1}) and MDMA decreased total object directed exploration, making it difficult to attribute the absence of discrimination to a specific memory impairment. Although MDMA had no influence on associative memory in the CER test, the highest dose of mephedrone significantly reduced freezing on re-exposure to the context used for conditioning, but had no effect on freezing produced by representation of the light and tone cue, suggesting mephedrone attenuated contextual but not cued association, which are mediated by different neuroanatomical substrates. Neither drug altered PPI (sensorimotor gating) assessed 30 min after the fifth injection. Taking all these data together, Shortall *et al.* (2013c) suggested that while mephedrone may impair cognitive performance, it is unclear whether this is due to a deficit in learning and memory and/or attention.

In rhesus monkeys, a pronounced improvement in visual-spatial memory and learning occurred after a 0.32 mg·kg^{-1} dose of both methamphetamine and mephedrone, although spatial working memory was not improved by either drug. This suggests mephedrone can improve spatial memory and learning in monkeys analogous with classical psychomotor stimulants (Wright *et al.*, 2012b).

Motbey *et al.* (2012b) also performed a battery of tests on rats given a higher dose of mephedrone (30 mg·kg^{-1}) once daily for 10 days but with a significant time gap (11–35 days) following the last dose and the start of the behavioural tests. Although repeated mephedrone did not cause any lasting changes in anxiety (elevated plus maze) or social preference, it caused a clear deficit in NOD 36 days after drug treatment. den Hollander *et al.* (2013) used a similar protocol to examine the lasting consequences of repeated mephedrone (30 mg·kg^{-1} twice daily for 4 days) in mice, examining anxiety (elevated plus maze), spatial working memory (T-maze spontaneous alternation), long-term spatial memory (Morris water maze) and depressive behaviour (tail suspension test) 2 weeks after injections. Anxiety and depression-related behaviours were unaffected by mephedrone, but consistent with Motbey *et al.* (2012b) mephedrone did reduce working memory.

In acute dose studies, low to medium doses of MDMA (≤10 mg·kg^{-1}) cause an anxiogenic response in rats in the x-maze, whereas higher doses (≥15 mg·kg^{-1}) tend to reduce anxiety-related behaviour (Ho *et al.*, 2004).

Abuse liability

It has been proposed that the predominant action of all cathinones on DAT is probably associated with a risk of addiction (Simmler *et al.*, 2013). This is consistent with common reports from recreational users that they became addicted or dependent on the drug (Dargan *et al.*, 2010). This risk contrasts with MDMA where users may suffer from some adverse events on acute withdrawal, but unequivocal reports of dependence or withdrawal are completely absent. Simmler *et al.* (2013) proposed that the twofold greater blood–brain barrier permeability of mephedrone over both methamphetamine and MDMA may produce a relatively greater reinforcing effect of the drug.

Aarde *et al.* (2013) showed that mephedrone supports i.v. self-administration in rats, resulting in consistent levels of drug intake from session to session and reward lever selectivity greater than 80% in most rats. Recently, Motbey *et al.* (2013) also showed that mephedrone robustly supported self-administration in rats. In contrast, MDMA is not readily self-administered by rats (De La Garza *et al.*, 2007) and produces considerable inter-individual heterogeneity in acquisition (Schenk, 2009; Colussi-Mas *et al.*, 2010; Bird and Schenk, 2013). The report of Aarde *et al.* (2013) supports the findings of Hadlock *et al.* (2011) that mephedrone supports self-administration in rats housed in high-ambient temperature conditions. Interestingly, high-ambient room temperature also increases MDMA self-administration (Cornish *et al.*, 2003).

Lisek *et al.* (2012) recently reported that mephedrone produced changes in conditioned place preference in rats and mice. The preference shift detected following mephedrone conditioning suggests that the drug displays rewarding properties consistent with a risk of abuse liability. However, this shift was only seen at a very high dose.

Intracranial self-stimulation that measures the behavioural effects of psychoactive compounds on brain reward circuitry was used by Robinson *et al.* (2012) to investigate the ability of mephedrone and cocaine to alter responding for electrical stimulation of the medial forebrain bundle in mice. Adult male mice with unipolar stimulating electrodes implanted in the lateral hypothalamus responded for varying frequencies of brain stimulation reward (BSR). The frequency that supported half maximal responding (EF_{50}), the BSR threshold and the maximum response rate were determined before and after saline, mephedrone (1, 3 or 10 mg·kg^{-1}) or cocaine at the same doses. Mephedrone produced a dose-dependent decrease in EF_{50}, threshold and the maximum response rate beginning 15 min after administration. Cocaine dose-dependently lowered the EF_{50} and threshold beginning immediately after administration, but did not affect maximum response rate. These results suggest that mephedrone, like cocaine, potentiates BSR, which the authors concluded may indicate its potential for abuse.

Drug interactions

Currently, very few studies have investigated possible interactions of mephedrone with other drugs. This is important as the combination of MDMA with other drugs, taken knowingly or unknowingly by recreational users, has been suggested to contribute to severe adverse events and possibly long-term neurotoxicity (Green *et al.*, 2012b). Furthermore, most mephedrone users admit previous or concurrent illicit use of MDMA (Carhart-Harris *et al.*, 2011; Moore *et al.*, 2013), so possible interactions of mephedrone with other psychoactive drugs, including MDMA, are likely. Our own preliminary study found that MDMA pre-exposure altered the subsequent temperature response to a challenge dose of mephedrone, suggesting cross-sensitivity of some functional responses in rats (S.E. Shortall *et al.*, unpubl. data). The study of Angoa-Pérez *et al.* (2013) in mice demonstrated that while mephedrone alone failed to produce any neurotoxic damage, it did enhance methamphetamine-induced neurotoxicity in dopamine nerve endings. It also enhanced the neurotoxic effects of amphetamine and MDMA on dopamine neurons, suggesting that a potentially dangerous interaction might occur when mephedrone is taken with other recreational drugs. In a subsequent study, the same group found that mephedrone did not cause 5-HT toxicity in the hippocampus even when co-administered with methamphetamine or MDMA (Angoa-Pérez *et al.*, 2014).

The term 'bath salts', which seems prevalent in the U.S. recreational drug scene, often refers to some of the newer cathinone-related compounds that are now being distributed using names to hide the fact that they are intended for human ingestion (Baumann *et al.*, 2013a,b). The content of 'bath salt' powders can include mephedrone, methylone (3,4-methylenedioxymethcathinone; Figure 1) and 3,4-methylenedioxypyrovalerone (MDPV). The different pharmacology of these compounds at monoamine receptor sites (Simmler *et al.*, 2013) means that mixing them could have serious adverse effects in humans. Recently, for example, Cameron *et al.* (2013) showed that mephedrone and MDPV, due to their different actions at the dopamine nerve ending, might be expected initially to release dopamine and subsequently prevent its re-uptake via DAT. This combined action could have serious adverse effects on brain function.

Over the last few years, there has been increasing evidence that caffeine, an adulterant sometimes found in 'ecstasy' tablets and an ingredient in coffee, tea, and in many soft and 'energy drinks' such as Red Bull, enhances both the hyperthermia and the neurotoxicity induced in rats by MDMA raising concerns about its possible effects in humans (Vanattou-Saïfoudine *et al.*, 2012). We have now found that the mephedrone-induced decrease in rectal temperature was reversed by combined caffeine administration, and a sustained hyperthermia occurred which had not returned to baseline levels even at 120 min post-injection (Shortall *et al.*, 2013b). One possibility is that this elevation in temperature observed when both drugs are administered may explain the hot flushes sometimes reported by mephedrone users (Schifano *et al.*, 2011). Importantly, this drug combination did not appear to induce 5-HT neurotoxicity in the brain (Shortall *et al.*, 2013b) as occurs when caffeine is given with MDMA (Vanattou-Saïfoudine *et al.*, 2012).

Conclusions

As detailed in the Introduction, although recreational users have stated that the psychoactive effects of mephedrone are similar to those of MDMA, the preclinical studies detailed in this review make it clear that these two drugs have a rather different, albeit related pharmacology. Table 1 provides a subjective overview of the behavioural pharmacokinetic and pharmacological effects of mephedrone and MDMA in rodents indicating some key supporting references for each comparator; however, full information is provided in the text of this review. Mephedrone has some properties that suggest its adverse effect profile might be less than MDMA, but its use by humans still raises significant safety concerns.

On the positive side, mephedrone, at least when given at a dose to rats that may have translational relevance, does not appear to induce monoamine neurotoxicity or produce hyperthermia in the majority of investigations. However, hyperthermia did occur when mephedrone was combined with caffeine. Of note are the indications that mephedrone has a short plasma half-life in rats and probably in humans, which is probably the reason why many recreational users take repeated doses over a short period. This binge use may induce more severe adverse consequences. What is also becoming clear from preclinical studies is that mephedrone has high-abuse liability resulting from several pharmacokinetic and pharmacodynamic differences from MDMA. Firstly, it has high brain penetration, rapid metabolism and brain clearance all of which are likely to lead to an acute withdrawal phenomenon. This does not occur with MDMA which has slower brain penetration, metabolism and clearance, both in rats and crucially in humans. Secondly, its interaction with monoamine neurotransmitters is very different from that of MDMA with greater potency at DAT and causing more dopamine release, leading Simmler *et al.* (2013) to suggest that mephedrone acts as a mixed 'cocaine-MDMA' type drug having some properties of both compounds. The 5-HT releasing property produces the 'entactogenic' subjective effects desired by many users, but because of its ability to potently activate the noradrenaline and dopamine systems, mephedrone is also likely to have a high psychostimulant and abuse liability. In contrast, MDMA has greater potency at SERT than mephedrone, and its dopamine/5-HT release potency will produce positive mood with little psychostimulant effect. Self-administration studies in rats show that mephedrone, unlike MDMA, robustly supports this behaviour.

Emerging data suggest that, like amphetamine and its derivatives, the cathinones all have their own specific pharmacological profile. Consequently, the pharmacology of mephedrone reviewed here cannot be taken as a template for the properties of other illicit cathinone derivatives that are appearing. The medical problem is that recreational users of the newest compounds are acting as 'laboratory animals', because none of the drugs have undergone any thorough preclinical evaluation similar to those now being published on mephedrone.

Table 1

Comparator overview of some of the major properties of mephedrone and MDMA

Behavioural measurements	Mephedrone	MDMA
Locomotor activity[1–3]		
• Duration	↑	↑↑
• Magnitude	↑	↑
• 5-HT syndrome	✓	✓
• Sensitization to same drug	✓	✓
Body temperature[4–6]		
• Singly housed	↓	↓↓
• Group housed	↔	↑↑
• High-ambient temperature	↔↑[§]	↑↑↑
Drug self-administration[6]	↑↑↑	↑
Chemical measurements		
Pharmacokinetics[7,8]		
• Plasma half-life (h)	~0.4	~0.8
• Half-life similar to humans	✓	X
• Active metabolites	**?**	✓
• Brain penetration	↑↑	↑
Brain monoamine release *in vivo*[9–11]		
• 5-HT	↑↑↑	↑↑↑
• Dopamine	↑↑	↑
Brain monoamine content at 60 min[12–13]		
• 5-HT	↓	↓↓↓
• Dopamine	↑	↑
Brain neurotoxicity[14–16]		
• 5-HT in rats	**?*** [17]	✓
• Dopamine in mice	–	✓
Monoamine uptake inhibition (IC_{50})[18,19]		
• SERT	422	125
• DAT	762	1009
• NET	487	450
Monoamine release (EC_{50})[20–22]		
• SERT	122	39
• DAT	51	42
• NET	58	53

Data compiled to compare the behavioural effects and pharmacokinetic measurements were derived from studies using a single acute s.c. or i.p. injection of either drug (typically 1–10 mg·kg^{-1} but up to 30 mg·kg^{-1} for mephedrone) in rats and where available in mice, but in some microdialysis studies i.v. administration was used. Studies examining a neurotoxic effect have used multiple doses (either on the same day or over a few weeks) typically administering 10–30 mg·kg^{-1} s.c. or i.p. Arrows show relative size of behavioural response, either increase (↑) or decrease (↓), ↔ indicates no significant change from control. Check mark (✓) shows that a response or change occurs, dash (–) means change not seen, and X means the value is different from humans. IC_{50} and EC_{50} values are both reported in nM and taken from Baumann *et al.* (2013a).

*Indicates that one of five studies reported neurotoxic damage. Combinations of other drugs with either MDMA or mephedrone and have been reported to, respectively, enhance or induce toxicity as detailed in the text.

[§]Indicates conflicting results. Key references supporting the synopsis for each parameter are indicated as a numerical superscript and below, but further references are provided in the relevant section of the review: [1]Kehr *et al.* (2011), [2]Lisek *et al.* (2012), [3]Green *et al.* (2003), [4]Shortall *et al.* (2013a, b), [5]Miller *et al.* (2013), [6]Docherty and Green (2010), [7]Baumann *et al.* (2009), [8]Martínez-Clemente *et al.* (2013), [9]Kehr *et al.* (2011), [10]Wright *et al.* (2012a), [11]O'Shea *et al.* (2005), [12]Motbey *et al.* (2012a), [13]Colado and Green (1994), [14]Shortall *et al.* (2013a, c), [15]Motbey *et al.* (2012a), [16]Green *et al.* (2003), [17]Hadlock *et al.* (2011), [18]Martínez-Clemente *et al.* (2012), [19]Baumann *et al.* (2013a), [20]Nagai *et al.* (2007), [21]Baumann *et al.* (2011), [22]Simmler *et al.* (2013).

Conflict of interest

The authors declare no conflicts of interest.

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