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In: Current Pharmaceutical Biotechnology, 11 (5), 421-433, 2010

<http://www.benthamdirect.org/pages/content.php?CPB/2010/00000011/00000005/0003G.SGM>

To refer to or to cite this work, please use the citation to the published version:

Stove, Christophe; De Letter, Els; Piette, Michel; Lambert, Willy (2010). Mice in Ecstasy: Advanced Animal Models in the Study of MDMA. Current Pharmaceutical Biotechnology, 11 (5), 421-433.

Review: mice in ecstasy: advanced animal models in the study of MDMA

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ABSTRACT

The party drug 3,4-methylenedioxymethamphetamine -better known as MDMA or *ecstasy*- has numerous effects on the human body, characterized by a rush of energy, euphoria and empathy. However, also a multitude of toxic/neurotoxic effects have been ascribed to MDMA, based upon case reports and studies in animals. Given the intrinsic difficulties associated with controlled studies in human beings, most of our insights into the biology of MDMA have been gained through animal studies. The vast majority of these studies utilizes a pharmacological approach to elucidate the mechanisms by which MDMA exerts its effects. Advances in genetics during the last decade have led to the development of several mouse models (transgenic or knockout) that have greatly contributed to our understanding of MDMA biology. This review provides an overview of these genetically modified animal models, in the light of some characteristic effects of MDMA, e.g. hyperlocomotion, neurotoxicity, hyperthermia, behaviour or rewarding. Without a shadow of a doubt, the next decade will bring many more advanced animal models, such as mice with site-specific deletion or rescue of genes and more genetically modified rat models. These models will further improve our knowledge on the pharmacology and toxicity of MDMA and, possibly, may assist in developing therapies coping with potential damage in abusers of MDMA and other drugs, as well as in patients suffering from specific neuronal pathologies.

MDMA in advanced animal models

Keywords: ecstasy, knock-out, MDMA, 3,4- methylenedioxymethamphetamine, mouse models, review, transgenic

I. Introduction

In the late 1980s, the psychoactive recreational drug *ecstasy* was associated with the *rave* culture and, since the 1990s, became more widely available in many dance clubs and other venues. Still now, *ecstasy* is most often consumed by youngsters during the weekend at *techno* and *rave* dance parties [1]. Whereas in past years, *ecstasy* tablets may have had singularly, or in combination, MDMA (3,4-methylenedioxymethylamphetamine), MDEA (3,4-methylenedioxyethylamphetamine) or MDA (3,4-methylenedioxyamphetamine) as the major psychoactive constituents, the majority of more recently seized tablets sold as *ecstasy* do contain MDMA as the primary psychoactive component [2].

The unique pharmacological properties of MDMA result in behavioural effects that are distinct from those of both phenylalkylamine psychostimulants and classical hallucinogens (as described by L. Schmued in detail in this issue). Typically, the mood enhancing properties of MDMA are summarized in the 3 E's: Energy, Empathy and Euphoria, which are pretty well reflected by the name under which it was originally (and still is) "marketed": *Ecstasy*. In addition, these effects are often accompanied by post-drug anxiety and agitation [3]. The pharmacology of MDMA is distinct from that of amphetamines and other stimulants (e.g. cocaine) in the fact that it not only produces an increase in drive and energy (encouraging and allowing users to dance for long periods), but also a sense of warmth and empathy with others, and therefore it is also referred to as an 'empathogen' or 'entactogen' [1, 4]. At the cellular level, MDMA acts by influencing the balance of monoamine neurotransmitters in the brain, with as main action a disturbance of the homeostasis of serotonin (5-hydroxytryptamine, 5-HT) and dopamine (reviewed by L. Schmued elsewhere in this issue). 5-HT is a modulatory neurotransmitter that acts throughout the brain and has been shown to play a role in a number of behaviours, including sleep, appetite, thermoregulation, reward, locomotion and mood. A prime effect of MDMA is the release of 5-HT from presynaptic nerve terminals, which then may act on nearby 5-HT receptors. Central in this effect is the modulation by MDMA of a major regulator of synaptic availability, the 5-HT transporter (5-HTT). This transporter is the prime target of most antidepressant and anxiolytic drugs, being (selective) serotonin reuptake inhibitors, ((S)SRIs) [5]. In fact, the basis for the involvement of 5-HTT in MDMA neurotoxicity was laid by showing that antidepressants like fluoxetine or fluvoxamine could prevent serotonergic neurotoxicity in rats [6-8]. Thus, MDMA can be described as an indirect agonist at most 5-HT receptors. Its empathogenic properties are thought to be due to the release of 5-HT in the brain, of which MDMA causes the release to a much greater extent than other psychostimulants (such as cocaine or amphetamine) [9, 10]. In addition to its indirect effect at 5-HT receptors, MDMA may also exert some effects by acting directly at 5-HT receptors [11]. More specifically, its action at 5HT_{2A} receptors has been associated with its hallucinogenic properties [9, 10]. Similar to other psychostimulants, MDMA treatment also results in release of the neurotransmitter dopamine, this release being more prominent at higher doses, though still remaining less pronounced when compared to other psychostimulants.

The majority of users consider their use of *ecstasy* as 'recreational', rather than viewing it as a drug for daily or dependent use [12-14]. Based upon data from users and presentations to emergency departments, it is clear that *ecstasy* users frequently also use other drugs and even combine *ecstasy* intake with the intake of alcohol, nicotine/tobacco, cannabis, ketamine, gamma-hydroxy butyric acid (GHB), cocaine or amphetamine [15-19]. In addition, pills sold as *ecstasy* do not only show a huge spread in the amount of MDMA being present,

but may contain other substances instead or in addition [2]. As a consequence, it is often difficult to attribute (toxic) effects in ‘real-life’ cases solely to *ecstasy* [20]. Irrespective of the debate about *how harmful ecstasy exactly is* [21], it is a fact that there is a wealth of literature available supporting the harmful effects that have been associated with the intake of MDMA [8]. These can be divided into the indirect effects, which are associated with behaviours in which the users may engage, such as risk-taking or prolonged energetic *raving*, and the direct toxic effects, especially seen at higher doses. The latter may range from relatively mild or subtle (e.g. vomiting, nausea, headache and impaired cognitive function) to potential life-threatening (e.g. cardiovascular collapse, severe hyperthermia, multi-organ failure) and from acute to long-term (related to neurotoxicity) (reviewed by [8]). Tolerance to some of the psychological actions of MDMA has been reported in some human users of high doses of the drug [22], which may result in taking even higher (more toxic) doses to get the desired effect. Important to note, however, is that adverse effects of MDMA are not restricted to high-dose-induced neurotoxicity. Studies in mice and rats have shown that low doses, which did not cause traceable neurotoxicity, could also induce deficits in e.g. learning and cognitive function (see further). Several contributing factors have been put forward with respect to the (neuro)toxic responses induced by MDMA, including, amongst others, hyperthermia and metabolism (of both MDMA and neurotransmitters) [8]. Given the non-linear pharmacokinetics of MDMA, it is difficult -or even almost impossible- to make a direct correlation between its plasma concentration and its (toxic) effects [23]. In addition, oxidative metabolism of MDMA gives rise to products with higher toxicity than MDMA itself. This implies that the rate of generation and/or elimination of these metabolites -which differs between and even within species- may determine the severity of the toxic effects. Despite the fact that there is more compelling evidence for MDMA-induced neurotoxicity in rodents than in humans, it is unlikely that the human brain is less sensitive than the rodent brain to a neurotoxic insult [8]. The scientific literature about MDMA intoxications provides a heterogeneous picture, with multiple case reports, detailing primarily acute complications -including death- and showing a wide range of plasma and tissue concentrations (reviewed by E.A. De Letter *et al.* elsewhere in this issue). Given the inherent limitations of observations made in ‘real-life’ *ecstasy* users and given the relatively limited availability of clinical studies in humans, most of our knowledge on the mechanisms of action of MDMA has been gathered from studies on animals. Most of these studies have been conducted in rats and mice, although also e.g. rabbits, dogs and non-human primates have been used. Since it is impossible to cover all these studies within this review, we will focus here especially on studies using genetically modified mice. For more detailed information on other animal studies and the other aspects of MDMA, the reader is referred to other reviews in this special issue, as well as to the recent extensive in-depth review by Capela *et al.* on the molecular and cellular mechanisms of ecstasy-induced neurotoxicity [8].

II. Essentials from studies performed in (genetically modified) rodents

The majority of our insights about the action of MDMA has been gained by the administration of pharmacological agents (e.g. agonists or antagonists at certain receptors) and by evaluating whether these had an impact on a certain effect of MDMA. Although the value of these studies should not be underestimated, these studies inherently suffer from some major drawbacks. First of all, most (if not all) pharmacological agents only have a limited specificity, which often makes it difficult to draw definitive conclusions about the implication of a specific receptor subtype in a given effect. Second, these pharmacological agents have an impact on the organism as a whole, which may render it difficult to discriminate between effects or effectors. Typical examples include pharmacological agents, which, in addition to their effect on the brain, may also influence MDMA metabolism in the liver or which, as such, already have an impact on body temperature.

Recently, multiple studies with genetically modified mice -transgenic or knockout (KO)- have complemented the studies with pharmacological agents and have thus improved our biological insight into the action of MDMA. Obviously, also in these studies, several limitations have to be kept in mind [24]. **First**, and most importantly, differences between animals (more specifically mice) and humans usually hamper some conclusions. **Second**, administration in the vast majority of these studies was by intraperitoneal injection of high dosages, which may not only lead to a difference in kinetics, but may as well result in a different profile of (toxic) metabolites. Although this acute administration differs substantially from the human situation, in which - predominantly oral- use may extend over weeks, months, or even years, it may be related to the final outcome, being neurotoxicity. **Third**, whereas several research groups have used racemic mixtures (better resembling the 'real-life' situation), others have used S(+)-MDMA, which is a more potent releaser of monoamines and was shown to have higher neurotoxicity. **Fourth**, strain differences may be an underlying cause of (at least) some conflicting findings, with different strains showing different sensitivities to (the toxic effects of) MDMA. **Fifth**, pharmacokinetics (including metabolism) and pharmacodynamics may differ substantially between different (and even within the same) species, which is of high relevance given the potential (toxic) effects of metabolites. **Lastly**, specifically holding true for genetically modified organisms, one has to bear in mind that developmental adaptations and/or redundancy (compensation by other proteins) may complicate the interpretation. With respect to this, genetically modified mice may be regarded sometimes as models of a disease state rather than as pharmacological models.

In addition to the limitations associated with projecting animal studies to humans, the complex neurochemical profile induced by MDMA has to be taken into account, with reported increases in the synaptic concentrations of 5-HT, dopamine, but also of norepinephrine, gamma-amino butyric acid (GABA) and acetylcholine [8]. The foremost important target of MDMA is the 5-HT system, which readily can be deduced from its higher affinity for the 5-HT transporter (5-HTT) than for the dopamine transporter (DAT) and from its ability to induce a more potent release of 5-HT than of dopamine [25-27]. Yet, also the dopamine and other systems have been shown to play a role (from modulatory to prominent) in certain effects of MDMA. In this context it should also be emphasized that several effects, such as drug reward, are typically the result of mechanisms that are inherently polygenic and involve the action of multiple neurotransmitters, acting to a different degree, depending on the circumstances [28]. Referring to all the above-mentioned possible confounding factors and the complex profile induced by MDMA, in addition, it should be kept in mind that mice

as such already differ substantially from other species regarding the pattern of MDMA-induced neurotoxicity. While rats and non-human primates (as well as humans) show primarily serotonergic neurotoxicity, dopaminergic neurotoxicity is also observed in mice [29, 30]. In the following section, an overview will be given of different models (primarily murine) that have been used to study many of the above-mentioned (toxic) effects of MDMA. As readily mentioned above, this review primarily aims at providing an overview of studies using genetically modified mice, rather than covering all described effects of MDMA. Although obviously all the effects of MDMA are inherently interconnected, the following sections have been organized arbitrarily according to several prototypical aspects of MDMA that have been investigated in these studies, including e.g. hyperlocomotion, hyperthermia, neurotoxicity or rewarding.

II.1. Pharmacokinetics and pharmacodynamics

In humans and rats, the main metabolism of MDMA occurs in liver, with a prominent role for CYP2D6 (orthologous to CYP2D1 in rat strains) [23, 31]. Although not genetically modified *per se*, the dark agouti rat strain, which lacks this enzyme, may mimic a ‘poor metaboliser’ in humans. MDMA administration to this strain leads to higher MDMA brain concentrations [32]. In addition, MDMA is known to auto-inhibit its metabolism, which explains its non-linear kinetics, exemplified by the fact that following high doses or after a second dose shortly after a first one, plasma concentrations increase disproportionally [23, 33, 34]. Despite these observations, no convincing link could be shown thus far between CYP2D6 genotype and MDMA intoxications [35-37]. This lack of association may be related to the nature of the metabolites, which have a higher (neuro)toxic potential than MDMA itself [38, 39]. Thus, metabolism of MDMA can be considered as a double-edged sword: a slower metabolism results in higher MDMA plasma concentrations, whereas faster metabolism results in a higher formation of toxic metabolites.

Phosphoglycoproteins (P-gps), encoded by a family of multidrug-resistant genes (*MDR1* and *MDR3* in humans and *mdr1a*, *mdr1b* and *mdr2* in mice), function by transporting molecules across membranes (including the blood-brain barrier) by acting as ATP-dependent drug efflux pumps [40]. In doing so, they provide cells with drug resistance, which may be favourable, but at the same time may impose problems with respect to therapeutic drugs. Mice defective in *MDR1a* have been used to study the role of P-glycoproteins in the neurotoxicity of certain drugs, including methamphetamine and MDMA [41]. While for methamphetamine there is evidence that the presence of *MDR1a* may provide protection against depletion of dopamine and dopamine transporters, the effects of MDMA are not inhibited in *mdr1a* *-/-* mice. A certain facilitation of MDMA passage through the blood-brain barrier in the presence of *MDR1a* in wild-type (wt) mice was even observed, with *mdr1a* *-/-* mice being more resistant to MDMA-induced reductions in dopamine and dopamine transporter expression, at least at certain dosages (5-10 mg/kg) [41]. This result, based upon an evaluation 1 week post-dose, suggests that *MDR1a* could be considered as a potential target for MDMA abuse treatment, since a candidate drug inhibiting *MDR1a* might limit MDMA’s acute and neurotoxic effects. However, arguing against an important role for *MDR1a* were the findings by Upreti and Eddington, showing that *mdr1a* wt and *-/-* mice did not show significant differences in brain concentration of MDMA or its active metabolite MDA 0.5 and 4h following a 5 mg/kg i.p. injection of MDMA [42]. Finally, Scheidweiler and colleagues, administering 10 to 40 mg/kg to *mdr1a* wt or *-/-* mice found differences in dihydroxyphenyl acetic acid (DOPAC) / dopamine ratios in striatal specimens from treated mice, which depended on the time point of evaluation and the dosage applied [43]. Also here, striatal concentrations of

MDMA and its metabolite MDA were not higher in wt than in *mdr1a* $-/-$ mice, suggesting that altered MDR1a-mediated transport of MDMA does not account for the observed differences of MDMA's effects on DOPAC/dopamine ratios between *mdr1a* $+/+$ and $-/-$ mice. Alternative mechanisms or compensatory changes in protein expression/activity in *mdr1a* $-/-$ mice could account for the observed inter-strain differences on dopamine turnover.

II.2. Hyperlocomotion

Similar to its energetic effect in humans, MDMA induces hyperlocomotion when administered to rats or mice. The mechanisms underlying this MDMA-induced hyperactivity have been the subject of multiple studies, which have applied a wide variety of genetically modified mice, pointing at the involvement of many different systems.

II.2.1. The serotonergic and closely related systems

MDMA effects on locomotor activity are primarily mediated by serotonergic activation. Low dosage (3-10 mg/kg) MDMA-induced hyperactivity in mice is (virtually) completely blocked by deletion of the genes for either the 5-HT_{1B} receptor [44, 45], the 5-HT_{2B} receptor [46] or the 5-HT transporter (5-HTT) [47]. With respect to the involvement of other 5-HT receptors in MDMA-induced hyperlocomotion, rat studies have suggested a lack of involvement of the 5-HT_{1A}R [48], while the 5-HT_{2A} and the 5-HT_{2C} receptor may have a facilitatory and inhibitory influence, respectively [49]. In contrast to wt mice, 5-HT_{2B}R $-/-$ mice do not show increased extracellular levels of 5-HT or dopamine following a 10 mg/kg dosage of MDMA [46]. In addition, the uptake activity of the 5-HT and dopamine transporters were unaltered in these mice, suggesting that the 5-HTT-dependent release of 5-HT requires the presence of a functional 5-HT_{2B}R. MDMA has been shown to require 5-HTT for releasing 5-HT [50, 51]. Indeed, 5-HTT $-/-$ mice, which readily have higher extracellular 5-HT levels in the prefrontal cortex than wt controls, did not show a further increase following MDMA stimulation (10 mg/kg). These mice lack the locomotor-stimulating effects of MDMA, with an intermediate effect seen in 5-HTT $+/-$ mice [47]. In contrast, amphetamine-induced (i.e. dopaminergic) hyperlocomotion is maintained in the 5-HTT $-/-$ mice. Apart from the dopaminergic system, described further, several other receptor systems appear to be modulating the 5-HT-dependent MDMA-induced hyperlocomotion.

- Compan and colleagues [52], using 5-HT_{1B}R KO mice, have suggested a role for enkephalins in MDMA's locomotor effects. They found that, 3 hours following a 10 mg/kg challenge, 129/Sv wt, but not KO mice, showed less enkephalin immunoreactivity in the globus pallidus, likely reflecting increased enkephalin release. This finding of increased 5-HT_{1B}R-mediated enkephalin release, together with the failure of MDMA to promote hyperactivity when combined with naloxone, led them to suggest a role for opioid signalling in MDMA-induced hyperlocomotion.
- Also the cannabinoid receptor system has been shown to modulate several pharmacological responses following acute MDMA administration. Whereas MDMA-treated cannabinoid receptor (CB1R) wt mice show increased locomotor activity, elevated body temperature, and anxiogenic-like (open field avoidance) responses, these responses are all less pronounced or even absent in CB1R $-/-$ mice. These results are likely related to the impaired serotonergic negative feedback in CB1R $-/-$ animals, which

under basal conditions readily show elevated extracellular 5-HT and reduced 5-HTT in brain areas like the prefrontal cortex [53].

- The MDMA-induced increase in locomotion, observed in wt mice, was less pronounced in histamine H3 receptor (H3R) $-/-$ mice, pointing to a functional interaction between histaminergic and serotonergic neurons which modulates the locomotor effects of MDMA [54].

Higher dosages of MDMA (30 mg/kg) did induce hyperactivity in both 5-HT_{1B}R KO 129/Sv and HT_{2B}R KO 129 Sv/PAS mice [55]. Sensitization to the locomotor-stimulating effects of MDMA, which was investigated in the 5-HT_{2B}R KO model, was absent at low-dose, but present at high-dose MDMA. The occurrence of locomotor stimulation (and sensitization) at high doses of MDMA, in the absence of HT_{1B}R or HT_{2B}R, might be owing to a delayed increase in dopamine release. Indeed, while administration of 30 mg/kg to 5-HT_{2B}R $-/-$ mice still didn't elevate extracellular 5-HT in the nucleus accumbens, extracellular dopamine levels did show an increase [55]. This suggests that the high-dosage MDMA-induced 5-HT release requires the presence of a functional 5-HT_{2B}R, while MDMA-induced dopamine release appears to be partially independent of the 5-HT_{2B}R. This dopamine release may thus explain the hyperlocomotion and locomotor sensitization observed at high doses of MDMA in 5-HT_{2B}R $-/-$ mice.

II.2.2. The nNOS system

Sensitization to the locomotor-stimulating effect of MDMA has also been evaluated in mice lacking neural NO synthase (nNOS $-/-$) [56]. These mice lack a locomotor response following a 5-day challenge with methamphetamine, but, in contrast, do not show a reduction in MDMA-induced hyperlocomotion following a 5-day challenge with 10 mg/kg MDMA [56], showing that nNOS is not required for MDMA-induced hyperlocomotion. Although these mice initially showed sensitization (with progressively higher locomotor activity during the 5-day challenge period), this sensitization towards MDMA-induced hyperlocomotion was no longer apparent when the mice were challenged 40 days later. This suggests that, whereas the induction of sensitization may primarily involve (nNOS-independent) 5-HT neurotransmission, the persistence of sensitization may depend on an intact nNOS system, possibly modulating dopamine neurotransmission.

II.2.3. The dopaminergic system

Well-defined tests for locomotor activity and locomotor patterns offer the possibility of scoring more subtle changes in behaviour, induced by psychostimulants, making it possible to score the qualitative aspect of the MDMA-induced locomotion. Using such systems, MDMA has been shown to induce locomotor activity (increased path length), with as peculiarities, increased path linearity (better predictable, straight paths) and increased repetition of specific paths (stereotypy; perseverative thigmotaxis), with mice typically running in straight lines around the periphery of an open test field. As discussed above, several studies have shown a prominent role for the 5-HT system in the hyperlocomotory effects of MDMA. This is exemplified by 5-HT_{1B}R $-/-$ mice treated with 30 mg/kg MDMA, in which the stereotypical aspect of hyperlocomotion was less than or equal to wt mice: MDMA-treated KO mice enter the open field more and have more curves and turns in their paths [45]. However, different studies using pharmacological agents have also shown that the dopamine receptor system is implicated in MDMA-induced locomotion and motor patterns, especially at higher doses. The precise involvement of dopamine receptors in this effect has been evaluated in wt, dopamine D1, D2 and D3 receptor

KO C57BL/6J mice that had been treated with MDMA [57]. Overall, gene deletion of D1, D2 or D3 has only moderate effects on MDMA-induced hyperlocomotion, highlighting the importance of non-dopaminergic systems for the effect of MDMA on locomotor activity. In general, modulation of the effect of a 20 mg/kg dosage of MDMA in these KO mice only became apparent at a later stage of the testing phase (40 min post-injection). This is in line with the observation that in 5-HT_{1B}R KO mice, which are refractory to MDMA hyperlocomotion at low dosages, administration of high MDMA dosages does result in a delayed hyperactivity [45]. More specifically, D1 and D2 KO mice exhibited respectively greater and reduced MDMA-induced hyperactivity (path length), as compared to wt mice. Of note are the differences seen in the MDMA-induced locomotory patterns in these mice: whereas D1 KO mice showed an increase in linear paths, D2 KO mice lacked the typical repetitiveness in their paths, as compared to wt mice. Changes in D3 KO mice were less pronounced, although female (but not male) D3 KO mice displayed slightly reduced MDMA-initiated hyperlocomotion [57].

Similar to the serotonergic system, where the 5-HT transporter (5-HTT) is a major regulator, the intensity and duration of dopamine signalling is under the major control of the dopamine transporter (DAT). Mice with a DAT (*Slc6A3*) gene deletion exhibit a chronic hyperdopaminergic state, presenting with a hyperactive phenotype, characterized by locomotor hyperactivity and a perseverative, stereotypical locomotor pattern at the periphery of an open field [58-60]. In fact, this phenotype resembles the one seen upon treatment of wt mice with MDMA. Paradoxically, despite the fact that MDMA increased locomotor activity in wt mice, it attenuated locomotor hyperactivity in DAT ^{-/-} mice [61]. This paradoxical calming effect is similar to the one observed following treatment of DAT ^{-/-} mice with other psychostimulants, which has been shown to involve serotonergic neurotransmission [59]. Likely, the treatment of DAT ^{-/-} mice with psychostimulants results in partial restoration of a correct balance between serotonergic and dopaminergic neurotransmission. This balance, which is required for normal motor activity, is disrupted in both non-treated DAT ^{-/-} mice and in wt mice following treatment with MDMA. Contrasting with its attenuation of hyperactivity in DAT ^{-/-} mice, is the potentiation by MDMA of other discordant behaviours of these mice. Following treatment with MDMA, DAT ^{-/-} mice showed exaggerated locomotor stereotypy (as evidenced by the occurrence of highly predictable, very straight paths and the avoidance of entering the central area of a testing chamber). The contrasting effects of MDMA on locomotor hyperactivity and locomotor diversity of DAT ^{-/-} mice indicate that different mechanisms underlie these effects. This is in line with i) the distinct phenotype that was observed in MDMA-treated D1 and D2 receptor KO mice (mentioned above) [57] and with ii) the finding that both D1 and D2 antagonists reduced locomotor hyperactivity in DAT ^{-/-} mice, whereas only the D1 antagonist normalized the MDMA-induced perseverative, stereotypical patterns [60]. The DAT ^{-/-} mice have also been used to generate an acute mouse model of Parkinson disease [62]. DAT ^{-/-} mice have elevated extracellular dopamine and highly decreased intracellular dopamine stores, which renders them very dependent on the ongoing synthesis of dopamine. As a consequence, pharmacological blockade of dopamine synthesis results in acute severe dopamine depletion, accompanied by the transient appearance of Parkinson disease symptoms (e.g. akinesia, rigidity, body tremor, ptosis (droopy eyelids)). Remarkably, the compound found to be most effective in inducing forward locomotion and in counteracting akinesia and rigidity in DDD (dopamine-depleted DAT ^{-/-}) mice was MDMA (at high doses). This indicates that MDMA can affect movement control in a dopamine-independent manner. A similar “anti-parkinsonian” effect has been observed in rats, in which MDMA counteracted haloperidol-induced parkinsonism [63].

II.3. Neurotoxicity

II.3.1. Models focusing on serotonergic and dopaminergic systems

As readily mentioned above, it remains a challenge to directly compare the neurotoxicity of MDMA in animals and humans, given the differences that exist between animals (especially rodents) and humans in terms of the pattern of drug exposure (administration vs. abuse) and of the mechanisms and assessments of neurotoxicity [24]. Nevertheless, there is now plenty of evidence demonstrating the neurotoxicity of MDMA to dopaminergic and serotonergic neurons in rodents and its potential to cause cognitive impairments in humans [8]. The neurotoxic effects of MDMA are typically studied using administration of high dosages of MDMA and/or repeated administration (“binge” treatment) to animals, reflecting to some extent the repeated exposure to high doses of MDMA in heavy users. As readily mentioned above, this approach has obvious limitations, but nevertheless may provide useful information on the neurotoxicity exerted by MDMA. Delayed (i.e. being considered as neurotoxic) effects of sub-chronic MDMA on the 5-HT system were studied by Renoir et al., evaluating wt and 5-HTT $-/-$ C57Bl/6J mice, 4 weeks after a 20 mg/kg dosage regimen of MDMA (administered twice daily during 4 days) [64]. Evaluation of the firing rate of dorsal raphe nucleus serotonergic neurons indicated a significantly lower firing rate in MDMA-treated wt but not 5-HTT $-/-$ mice. In MDMA-treated wt mice this effect was linked to an apparent hypersensitivity of the inhibitory 5-HT_{1A}R and to a decrease of 5-HT levels in most brain areas. Consistent with other studies [65-68], this delayed limited (though significantly) lower 5-HT tone in the brain of MDMA-treated wt mice was linked to a decreased hippocampal cell proliferation and a depressive-like behaviour. The latter was evidenced by an increase in immobility of MDMA-treated wt mice in a forced swim test, whereas the already higher immobility in 5-HTT $-/-$ mice did not further increase following sub-chronic exposure to MDMA. These findings support the fact that MDMA, via its persistent negative effects on the 5-HT system, may contribute to the mood and cognitive disorders observed in MDMA users. The fact that also the neurotoxic effects that were observed in wt mice were abolished in 5-HTT $-/-$ mice, lends support to an important role for this transporter in the mechanism of MDMA neurotoxicity, possibly via mediating entry of MDMA and its metabolites into 5-HT nerve terminals. As mentioned above, neurotoxicity in mice is -dependent on the strain and the conditions used- considerably dopaminergic. In particular, mice develop long-lasting dopamine axon terminal damage, with dopamine cell bodies remaining intact [8, 29]. Consistent with the importance of the dopaminergic toxicity of MDMA in mice is the observation that DAT $-/-$ mice are resistant to the neurotoxic effects of MDMA. Indeed, evaluation seven days after application of a neurotoxic regimen of MDMA (4x 20mg/kg, every 2h) showed that DAT $-/-$ mice were refractory to the neurotoxic potential of MDMA, while all wt mice had succumbed [62]. Using cDNA microarray technology, Xie *et al.* identified metallothionein-I and -II as two proteins whose expression appeared to be linked to MDMA-induced toxicity at dopamine neurons [69]. Metallothioneins are cysteine-rich, low molecular weight, heavy metal (i.e. zinc) binding proteins that have been postulated to detoxify metals; to play a role in homeostasis of processes or proteins requiring zinc; and to be neuroprotective (e.g. by protecting against reactive oxygen species). Their expression is induced in the central nervous system in response to practically any harmful challenge. Double *Mt1/Mt2* $-/-$ mice have been described to be more sensitive to toxic metals and oxidative stress [70, 71]. Likewise, these animals were more sensitive to MDMA’s neurotoxic effects, as evidenced by larger dopamine deficits 1 week after a neurotoxic regimen of MDMA, with no significant depletion of 5-HT and no significant differences in MDMA-induced hyperthermia [69]. This effect may be related to an inability of these animals to

cope with the higher oxidative stress which is imposed on them following MDMA treatment. Conversely, both zinc administration to wt mice, which increases metallothionein expression, and administration of metallothionein-I and -II protein, provided (partial) protection against MDMA-induced dopamine deficits. In conclusion, these mice may serve as a model system with increased sensitivity to the neurotoxic (dopaminergic) effects of MDMA.

Swiss Webster mice were shown to serve as a possible model of selective dopamine and 5-HT neurotoxicity following stimulation with appropriate doses of different psychostimulants [72]. In contrast to methamphetamine and fenfluramine, which respectively affect the dopaminergic and serotonergic system in these mice, MDMA administration to these mice results in a marked depletion of both dopamine and DAT binding sites and of 5-HT and 5-HTT binding sites. The dual dopaminergic/serotonergic depletion by MDMA in these mice may serve as a useful model for a similar depletion that may develop in humans abusing both methamphetamine and MDMA.

II.3.2. Models focusing on metabolism and oxidative damage

Support for a role of oxygen-based radicals in MDMA-induced neurotoxicity came from studies by Cadet *et al.*, using transgenic mice carrying the complete sequence of the human copper-zinc superoxide dismutase (CuZnSOD) gene [73, 74]. This mouse model was used to assess lethal, subacute and long-term effects of MDMA. In contrast to wt mice, homozygous CuZnSOD transgenic mice showed resistance to the lethal effects of MDMA and did not show depletion of striatal DA or DOPAC 24 h or 2 weeks following single or multiple (3 x every 24 h) injection with MDMA (50 mg/kg). The resistance against lethality was smaller in heterozygous CuZnSOD mice, which also showed DA depletion at the 24 h time point following a single injection and small decreases in DA levels following multiple injections. These findings suggest that both the acute lethal and the subacute and long-term effects of MDMA involve the intracellular overproduction of superoxide radicals. In a follow up study by the same group [75], they found that administration of MDMA (4 x 20 mg/kg) to CD-1 wt mice caused marked decreases in CuZnSOD activity in the frontal cortex, caudate-putamen and hippocampus, along with decreased catalase and glutathione peroxidase activity and increased lipid peroxidation. These altered enzyme activities were not observed in homozygous CuZnSOD mice. In summary, these data suggest that CuZnSOD overexpression could protect mice against MDMA-induced overproduction of free radicals, both direct and indirect, via inhibiting the perturbation of antioxidant enzymes.

Several reports have described the formation of reactive oxygen species (ROS) in the brain of MDMA-treated animals [8, 29, 76, 77]. Superoxide radicals may react with NO, formed through the action of NO synthases such as neural NO synthase (nNOS), resulting in the formation of the highly reactive neurotoxin peroxynitrite (ONOO⁻). Dopaminergic neurotoxicity, induced by high doses of psychostimulants, such as methamphetamine, was shown to be abrogated in mice deficient in neuronal nitric oxide synthase (nNOS ^{-/-}) [78]. However, as no difference in depletion of 5-HT in wt and nNOS KO mice was observed following a high-dose regimen of MDMA, this suggests that nNOS does not play a role in this effect of MDMA [56]. Remarkably, this high-dose regimen still lowered striatal dopamine levels in nNOS ^{-/-}, while DAT binding sites remained unaffected. This discrepant finding might be related to the occurrence of dopamine toxicity, secondary to 5-HT neurotoxicity.

Monoamine oxidases (MAO) are enzymes, bound to the outer membrane of mitochondria, which catalyze the oxidation of monoamines, such as 5-HT, dopamine or norepinephrin [79]. While a central role for MAO-A in the metabolism of endogenous monoamines such as norepinephrin, dopamine and 5-HT is well-established, MAO-B, at least in basal conditions, does not seem to play a major role in monoamine metabolism [80, 81]. An opposite role for MAO-B in MDMA-induced dopamine and 5-HT depletion can be deduced from the study of Fornai *et al.* [82]. Using high-dose (2x 50 mg/kg) MDMA treatment of MAO-B deficient mice and evaluation one week later, these authors found that, as compared to wt mice, 5-HT depletion does not occur in these mice, while dopamine loss was unaffected or became even more pronounced in MAO-B knockouts. On the one hand this may be related to a reduced formation of MAO-B-generated toxic dopamine metabolites in serotonergic neurons, while, on the other hand increased formation of toxic dopamine metabolites may take place in dopaminergic neurons, where also MAO-A may be less functional because of inhibition by MDMA [83].

II.4. Hyperthermia

Hyperthermia, which is often fatal when extreme, is the most prominent adverse reaction clinically assessed in human MDMA intoxications. Hyperthermia following MDMA administration has been observed in a wide variety of species, from mice, rats, pigs and non-human primates to human beings [84]. However, at least in some species, this acute dose-dependent rise in body temperature was shown to be dependent on the environmental temperature. Studies on rodents have demonstrated hypothermia in rats and mice following MDMA administration at lower temperatures [85, 86]. This hypothermia, following MDMA exposure at lower ambient temperatures, does not occur in humans and primates. Hyperthermia in humans typically occurs when MDMA is used in the setting of “dance parties”, in which factors such as crowdedness, high activity, high ambient temperature and dehydration exacerbate the otherwise mild hyperthermic effect (temperature rise up to 0.4°C) of MDMA seen under laboratory conditions [34]. As a result, excessive and potential life-threatening hyperthermia (body temperature up to 43°C) may occur in humans. Obviously, under laboratory conditions it is difficult to mimic this requirement of a ‘proper’ setting for the hyperthermic action of MDMA. Using a wide variety of *in vivo* and *in vitro* approaches, multiple studies have demonstrated the participation of multiple receptor pathways in the hyperthermic action of MDMA. These include the 5-HT_{2A} receptor (for which MDMA may serve as a direct agonist [11]), the Dopamine D1 receptor and the adrenergic receptor (α 1 subtypes) [8]. Several genetically modified mouse models have been used to study the hyperthermic effects of MDMA.

- The cannabinoid receptor signalling system, which is, as mentioned above, linked to the serotonergic system, has been implicated by Touriño *et al.* in modulation of the hyperthermic response [87]. These authors demonstrated that the elevation of body temperature following the administration of MDMA to cannabinoid receptor CB1R $-/-$ mice was significantly lower than in wt animals.
- MDMA has been shown to have affinity for α 2 adrenoreceptors (ARs) and MDMA treatment of rats results in activation of ARs [11, 88, 89]. A remarkable effect on body temperature was seen upon MDMA treatment of α 2_AAR $-/-$ mice: whereas MDMA (20 mg/kg) resulted in hyperthermia in wt mice, these KO mice showed a biphasic response, with initial hypothermia followed by hyperthermia [90]. Support for the specificity of this peculiar finding came from the fact that a similar observation, though less pronounced, was made in MDMA-treated wt mice that had been pre-treated with a rather selective α 2_A adrenoreceptor antagonist. An important role for centrally released norepinephrin in MDMA-

mediated thermogenesis, acting via a double-edged sword of heat generation, was proposed by Mills *et al.* [91]. On the one hand, increased norepinephrin plasma levels may lead to a loss of heat dissipation through $\alpha 1$ AR-mediated vasoconstriction, while stimulation of $\alpha 1$ and $\beta 3$ adrenergic receptors may regulate a mitochondrial protein in skeletal muscle, uncoupling protein-3 (UCP-3). Mitochondrial UCPs act by “uncoupling” free energy stored in the mitochondrial electrochemical proton gradient from ATP synthesis by regulating an inducible, thermogenic proton leak pathway. The importance of this *facultative thermogenesis pathway* was nicely demonstrated in UCP-3 $-/-$ mice, which were almost completely refractory to the rise in body temperature induced by high doses of MDMA [92]. Moreover, these mice were protected from the potentially lethal effect, induced by the strong hyperthermic response to high doses of MDMA.

II.5. Cognitive and behavioural impairments

MDMA abusers may suffer from neuropsychiatric deficits, including memory impairments [93-95], with recent studies suggesting that these deficits may persist after the cessation of abuse [95, 96]. Investigation of the behavioural and cognitive outcome of the (neurotoxic) effects of MDMA in mice may lead to a better understanding of the long-term consequences following the abuse of MDMA. Most of the knowledge regarding the mechanisms underlying cognitive impairments has been gathered through the use of pharmacological inhibitors. Up to now, only one study using genetically modified mice has evaluated the long-term cognitive consequences of MDMA administration. Cannabinoid CB1 receptors (CB1R) have been implicated in learning/memory, and are highly expressed in the hippocampus, a region of the brain believed to have an important function in certain forms of learning and memory. Using male CD1 wt and CB1R knockout (CB1R $-/-$) mice, Nawata and colleagues investigated the effect of withdrawal from repeated MDMA in an object recognition test [97]. Similar to what had been observed by others in rats [98-100], wt mice showed impaired recognition memory upon MDMA withdrawal. This impairment was not seen in CB1R $-/-$ mice and in mice treated with a CB1R antagonist, suggesting that the activation of CB1Rs is involved in the appearance of cognitive impairment upon withdrawal from MDMA. As readily mentioned above, this effect may be related to the tight cross-talk that exists between the cannabinoid system (CB1R) and the serotonergic system [53].

Another well-known effect following treatment of rodents with MDMA is a decrease in exploratory behaviour (typically evaluated as rearings and nose pokes). Interestingly, the effect of MDMA on this behaviour can be uncoupled from its stimulatory effect on locomotor activity. This was demonstrated in 5-HT_{1B}R $-/-$ mice, which show reduced MDMA hyperactivity, but maintain MDMA-suppressed exploratory behaviour [45]. This suggests that the exploratory suppression by MDMA does not involve the 5-HT_{1B}R, which is in agreement with pharmacological studies in rats [101]. Kindlundh-Hogberg and colleagues investigated the effect of MDMA on the exploratory behaviour of C57/Bl6 mice overexpressing S100B, a glia-derived calcium-binding protein which is induced by serotonergic agents and has a role in neuronal plasticity [102]. Rearing activity of these mice was more strongly reduced than in wt controls following binge treatments with MDMA (3 x 5 mg/kg per day, every 7 days, during 4 weeks). In addition, S100B overexpressing mice showed a stronger anxiolytic response following binge treatments with MDMA. Important to note in this context is that the differences seen between wt and S100B overexpressing mice were not seen following acute treatment, but only after repeated treatment. The mechanism underlying these differences remains to be elucidated.

The 5-HT-releasing properties of MDMA have also been used in 5-HT_{1B}R $-/-$ mice to study habituation and prepulse inhibition (PPI) [103]. These measures of fundamental behavioural processes refer to respectively the decrease in response to repeated presentations of an initially novel and intense stimulus and to the reduction in startle amplitude when a weaker prestimulus (prepulse) precedes a strong startling stimulus. Deficits in PPI and habituation have been observed in a variety of neuropsychiatric disorders, including schizophrenia [104]. Using acoustic stimuli, MDMA (10 mg/kg) reduced the startle magnitude but had no effect on startle habituation, whereas it affected PPI in a strain dependent way, with decreased PPI in C57BL/6 mice and in the outbred strain ICR, but not in a 129/Sv substrain [105, 106]. This decrease in PPI following MDMA administration was still present in 5-HT_{1A}R $-/-$ mice, while 5-HT_{1B}R $-/-$ 129/Sv mice showed an increased PPI [105, 107]. These results suggest that, following MDMA-induced 5-HT release, the 5-HT_{1B}R exerts a negative effect on PPI, which is not longer present in 5-HT_{1B}R $-/-$ mice (explaining the increase in PPI following MDMA administration in these mice). In summary, studies evaluating cognition (memory), exploratory behaviour and fundamental behavioural processes (sensorimotor gating) have shown that MDMA negatively impacts these processes. Studies using genetically modified mice have demonstrated the contribution of the serotonergic system (5-HT_{1B}R) and the tightly linked cannabinoid system (CB1R) in these effects.

II.6. Rewarding

MDMA has been shown to have reinforcing properties in humans [108, 109]. Murine, rat and monkey experimental models have also shown the rewarding properties of MDMA [109-116]. These models typically make use of conditioned place preference (CPP) tests (which evaluate the preference of an animal for a chamber in which it previously received MDMA) and operant intravenous self-administration tests.

Studies in 5-HT_{2B}R $-/-$ 129 Sv/PAS mice and 5-HTT $-/-$ C57BL/6 mice have shown the requirement of the serotonergic system for the reinforcing effect of MDMA [50, 55]. In contrast to wt mice, 5-HT_{2B}R $-/-$ mice completely lacked a rewarding response (evaluated using CPP) following repeated administration of low dosages (10 mg/kg) of MDMA [55]. Importantly, 5-HT_{2B}R $-/-$ mice did not show the accumbal dopamine release observed in wt mice, with concomitant activation of extracellular regulated kinase (ERK) in dopamine D1 receptor expressing neurons. Similarly, 5-HTT $-/-$ mice lack the acquisition and maintenance of self-administration of MDMA that is observed in a certain percentage of wt mice in an operant intravenous self-administration test (though to a lesser extent than seen with cocaine) [50]. However, contrasting with the findings in the 5-HT_{2B}R $-/-$ mice, the ability of low-dose MDMA to increase extracellular levels of dopamine in the nucleus accumbens remained unaffected in the 5-HTT $-/-$ mice. The lack of a rewarding effect in the presence of dopamine release in 5-HTT $-/-$ mice suggests a specific involvement of the 5-HT system in mediating MDMA's reinforcing effect. Hereby a requirement may exist for a fast change in 5-HT levels rather than for high absolute levels of extracellular 5-HT, which are readily present in 5-HTT $-/-$ mice. Given these observations and given the fact that pharmacological approaches have also shown a role for the dopaminergic system in the reinforcing effect of MDMA [117], it is plausible that (disruption of) a delicate interplay between both systems is required for the MDMA reward. Consistent with this is the finding that unlike "low-dosage" MDMA, a high dosage (30 mg/kg) does lead to a rewarding response (and concomitant release of dopamine but not 5-HT) in 5-HT_{2B}R $-/-$ mice [55]. This different effect of MDMA, according to the dosage applied, may relate to the different pharmacological targets of MDMA, resulting in different behavioural effects. Thus, at low

dosage, MDMA may release 5-HT in a 5-HT_{2B}R-dependent way, which may eventually lead to dopamine release in the nucleus accumbens. At high dosages, this dependence on 5-HT (and hence on 5-HT_{2B}R) may be overcome, possibly because of a direct effect of MDMA on DAT.

Activation of CB1Rs has also been shown to be involved in the MDMA reinforcing effect. Similar to the failure of CB1R $-/-$ mice to establish cocaine, morphine and ethanol self-administration to an extent as wt mice did [118-120], the CB1R $-/-$ mice did not acquire the performance of MDMA self-administration [87]. However, these KO mice maintained their reaction to MDMA in a conditioned place preference test, similar to what had been observed in the response of these mice to cocaine [119, 121]. This loss of response of the CB1R $-/-$ mice in the self-administration test, but not the CPP test, suggests that the role played by the CB1R in the rewarding response does not lie in the primary rewarding response (scored by the CPP), but rather relies on brain circuits related to reward, motivation and long-term learning (scored by the self-administration test). This hypothesis fits with the finding that MDMA treatment did not induce differences in dopamine release between wt and CB1R $-/-$ mice, with both groups showing a similar increase in dopamine extracellular levels in the nucleus accumbens, which is known to be involved in the acute rewarding effects of psychostimulants [122].

A possible role for μ -opioid receptors in the rewarding properties of MDMA was investigated by Robledo *et al.* [123]. These authors applied the conditioning place preference paradigm onto μ -opioid receptor $-/-$ C57BL/6 mice, investigating whether these mice still had a preference for the compartment in which MDMA had been administered before. KO mice did not differ from wt, neither in terms of rewarding, nor in terms of MDMA-induced increase in extracellular dopamine in the nucleus accumbens. These results show that μ -opioid receptors are not necessary for the rewarding effects of MDMA. Using a similar conditioning place preference test, Okuda and colleagues found that also histamine H3 receptor knockout C57BL/6J mice (H3R $-/-$) did not differ from wt mice in their rewarding response to MDMA [54].

II.7. Various effects

II.7.1. Anorectic properties of MDMA

In line with the well-known potent anorectic properties of 5-HT-releasers, MDMA is known to have appetite suppressant properties, as has been observed in e.g. dogs, rats and mice [124, 125]. In starved 129/Sv mice, a 10 mg/kg MDMA dosage provoked a biphasic feeding response, with initial 1 hour hypophagia, followed by hyperphagia during the next few hours [124]. This MDMA-induced delay in feeding response was maintained in 5-HT_{1B}R $-/-$ mice, which do not longer show the typical MDMA-induced hyperactivity [124]. Two studies have shown a role for 5HT_{2C} and 5-HT₄ receptors in the appetite suppressant effects of MDMA [124, 126]. In a first study, the co-administration of RS102221, a 5-HT_{2C}R antagonist, prevented both the hypophagic and hyperlocomotion effects of MDMA [124]. In another study, Jean *et al.* elegantly demonstrated that the anorectic effect of MDMA involves upregulation of the satiety factor CART (cocaine- and amphetamine regulated transcript) in the nucleus accumbens, via a cAMP/PKA signalling pathway, following activation of 5-HT₄Rs [126]. In the absence of either 5-HT₄Rs (5-HT₄R $-/-$) or CART upregulation (following knockdown via intracerebral injection of siRNA), there was (partial) inhibition of MDMA-induced hypophagia. Since 5-HT₄R KO mice are less sensitive to stress, it is possible that the observed effects are the result of a diminished response to the “stressor” MDMA [126].

II.7.2. Narcolepsy

Psychostimulants have a role in the treatment of narcolepsy, a chronic sleep disorder characterized by excessive daytime sleepiness and disturbed nocturnal sleep. A mouse model for narcolepsy, generated by knocking out orexin, has been shown to have remarkable similarities to human narcolepsy, in which a dysfunctioning orexin/hypocretin system has been well-established now [127, 128]. As compared to wt mice, non-treated C57Bl/6 orexin deficient mice show a significantly reduced dopamine turnover and locomotor activity [129]. However, stimulation of locomotor activity following treatment of these knockout mice with MDMA was more prominent than in wt mice. Based upon the use of pharmacological 5-HT receptor inhibitors, a role for functional changes in 5-HT_{1A}-, 5-HT₂- and 5-HT₇ receptors has been suggested to underlie this effect in orexin deficient mice [129].

II.7.3. Cardiovascular effects

Using KO mice, the group of Docherty set off to investigate the role of α_2 _AAR (α_2 _D in rodents) in MDMA's vascular actions and its prejunctional actions in vas deferens [130, 131]. Reported cardiovascular actions of MDMA in humans include tachycardia and a (transient) rise in blood pressure (extensively described by K. Varner elsewhere in this issue)[108]. Similarly, MDMA administration (5 or 20 mg/kg) to C57Bl/6 mice resulted in an increased heart rate and an initial rise in arterial pressure ("pressor" response) [130]. In anaesthetised -but not in conscious- mice this initial pressor response is followed by a decrease in arterial pressure shortly thereafter ("depressor" response). MDMA-treated anaesthetised α_2 _AAR $-/-$ mice have a prolonged pressor response, with no depressor response occurring, at least not within the first 10 minutes after treatment. The absence of a depressor component in MDMA-treated anaesthetised α_2 _AAR KO mice suggests that these receptors act to shorten the duration of the pressor response. The prejunctional actions of MDMA in vas deferens, however were unaffected in the α_2 _AAR $-/-$ mice [131]. Functional replacement of the α_2 _AAR during development by another α_2 AR, possibly the α_2 _CAR, may account for this observation.

III. Discussion & conclusion

Together, the use of advanced animal models, in particular genetically modified mice, has significantly contributed to our understanding of the mechanisms underlying the (toxic) effects of MDMA. These models, together with models under development, may help to answer remaining questions and may assist in the development of therapies, aimed at ameliorating potential neuronal damage and cognitive impairment following abuse of MDMA and related substances. Up to now, only constitutive mouse models have been described, expressing or lacking expression of a given gene readily from early development on. Obviously, a wealth of information on the role of specific neurotransmitter systems in confined brain regions or in well-defined neuronal subpopulations can be gained using (inducible) tissue-specific knockout, transgenic and rescue strategies. Such models with temporally and/or spatially regulated gene disruption or overexpression offer a significant advantage over the typically used pharmacological approach, because they allow the study of the role of a particular protein in a particular cell population. Recently, 5-HTT knockout rats have been generated [132], which, upon comparison with 5-HTT KO mice show overall major similarities in multiple neurobehavioral domains [133]. Given the fact that MDMA-induced neurotoxicity in rats better resembles that observed in humans, important information may be gained using these rats. Whereas only few genetically modified rat models are currently available (with not a single publication describing treatment with MDMA yet), recent progress in the field of developing genetically modified (knockout) rats by optimized and/or newly available technologies will likely lead to the development of many more strains in the coming years [134, 135]. Alternatively, local knockdown of particular genes in certain brain areas via RNAi (by injection of siRNA's or viral vector-mediated) may be a complementary tool [136, 137]. The latter approach has, in addition to being less time-consuming, labour-intensive and expensive, the advantage that possible developmental compensation or adaptation may be overcome. However, a drawback is that complete knockdown *in vivo* is not feasible. Without a shadow of a doubt, the introduction of these advanced new tools and animal models -both mice and rats- will greatly progress our fundamental insights into the pharmacology and toxicity of MDMA in the upcoming decade.

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