Experimental Neurology 347 (2022) 113894

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Review article

Neurotoxicity of MDMA: Main effects and mechanisms

Giulia Costa [a,*], Krystyna Gołembiowska [b]

[a] Department of Biomedical Sciences, Section of Neuroscience, University of Cagliari, Cagliari, Italy
[b] Maj Institute of Pharmacology, Polish Academy of Sciences, Department of Pharmacology, 12 Smętna, 31-343 Kraków, Poland

ARTICLE INFO

Keywords:
Ecstasy
Excitotoxicity
Hyperthermia
MDMA metabolites
Mitochondrial dysfunction
Neurotoxicity
Neuroinflammation
Oxidative stress

ABSTRACT

Preclinical and clinical studies indicate that 3,4-methylenedioxymethamphetamine (MDMA; 'ecstasy'), in addition to having abuse potential, may elicit acute and persistent abnormalities of varying severity at the central level. Importantly, neurotoxic effects of MDMA have been demonstrated in experimental animals. Accordingly, central toxicity induced by MDMA may pose a serious harm for health, since MDMA is among the substances that are used for recreational purposes by young and adult people. This review provides a concise overview of recent findings from preclinical and clinical studies that evaluated the central effects of MDMA, and the mechanisms involved in the neurotoxicity induced by this amphetamine-related drug.

1. Introduction

The psychostimulant 3,4-methylenedioxymethamphetamine (MDMA; 'ecstasy') is an amphetamine-related drug that possesses abuse liability and is used for recreational purposes, especially among young adults. The European drug report, 2017 released from the European monitoring centre for drugs and drug addiction (EMCDDA) in 2017, reported that MDMA abuse is increasing and nowadays it is not only limited to people attending dance clubs and parties, as happened since the early 1980s, but includes a broad range of mainstream nightlife settings, including bars and house parties. These data confirm the findings by pioneering investigations by Siegel (1986), who reported that the majority of individuals taking MDMA were experimental users, who used the drug for a maximum of ten times and in social situations, and reported their use of MDMA to be driven by their curiosity about the drug and the expectancy of acute positive effects of the drug on the emotional state.

The positive and untoward effects of MDMA vary according to the dose, frequency and duration of the use. In general, low doses of MDMA taken in single occasions induce acute positive effects on the emotional state (Moratalla et al., 2017), that include euphoria, arousal, relaxation, increased sociability and closeness with others (Holze et al., 2020). Nevertheless, MDMA may also induce untoward effects whose manifestation and severity may be influenced by a multitude of environmental and pharmacokinetics factors (Hall and Henry, 2006). Although the acute untoward effects of MDMA are well characterized in both humans and experimental animals (Table 1), several controversies exist about the long-term noxious effects elicited by the repeated exposure to MDMA in heavy users, particularly with regard to the effects that involve the manifestation of neurotoxicity in the central nervous system (CNS) (Table 2). In fact, different mechanisms and factors appear to be involved in the long-term noxious effects of MDMA, as indicated by findings in experimental animals demonstrating that the appearance of these effects, in particular neurotoxicity, is influenced not only by drug-

Abbreviations: α-MeDA, α-methyldopamine; CNS, central nervous system; COMT, catechol-*O*-methyltransferase; CPu, caudate-putamen nucleus; CSF, cerebrospinal fluid; DA, dopamine; DAergic, dopaminergic; DAT, DA transporter; 5,7-DHT, 5,7-dihydroxytryptamine; EMCDDA, European monitoring centre for drugs and drug addiction; GFAP, glial fibrillary acidic protein; HHA, 3,4-dihydroxyamphetamine; HHMA, 3,4-dihydroxymethamphetamine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; iNOS, inducible form of nitric oxide synthase; L-DOPA, 3,4-dihydroxy-*l*-phenylalanine; MAO, monoamine oxidase; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; nNOS, neuronal form of nitric oxide synthase; PET, positron emission tomography; PFC, prefrontal cortex; PD, Parkinson's disease; ROS, reactive oxygen species; RNS, reactive nitrogen species; SERT, 5-HT transporter; SNc, substantia nigra pars compacta; SOD, superoxide dismutase; SOD1, cytoplasmatic isoform 1 of superoxide dismutase; SOD2, mitochondrial isoform 2 of superoxide dismutase; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase; VTA, ventral tegmental area.

* Corresponding author at: Department of Biomedical Sciences, Section of Neuroscience, University of Cagliari, Building A, Monserrato University Campus, SP 8, Km 0.700, 09042 Monserrato, Italy.

E-mail address: gcosta@unica.it (G. Costa).

https://doi.org/10.1016/j.expneurol.2021.113894
Received 17 June 2021; Received in revised form 1 October 2021; Accepted 8 October 2021
Available online 13 October 2021

Table 1
Summary of the acute central untoward effects elicited by 3,4-methylenedioxymethamphetamine (MDMA) in humans and experimental animals. For a more complete list concerning central and peripheral effects of MDMA please refer to Green et al., 2003; Moratalla et al., 2017, Costa et al., 2020.

	Acute untoward effects
Humans	• Agitation; • Appetite suppression; • Bruxism; • Difficult to concentrate; • Dry mouth; • Headache; • Hyperthermia; • Nausea.
Experimental animals	• Hyperactivity; • Hyperthermia; • Serotonin behavioral syndrome (enhanced locomotor activity, reciprocal forepaw treading, head weaving, piloerection, hind limb abduction, proptosis, ataxia, and subsequent dose-dependent convulsions and death).

Table 2
Summary of the long-term central untoward effects elicited by 3,4-methylenedioxymethamphetamine (MDMA) in humans and experimental animals. For a more complete list concerning central and peripheral effects of MDMA please refer to Moratalla et al., 2017, Aguilar et al., 2020; Costa et al., 2020.

	Long-term untoward effects
Humans	• Anxiety; • Cognitive deficits (i.e.: verbal learning, attention, and working memory); • Depressed mood; • (Reversible?) Reduction of SERT density in parietal, temporal and occipital lobe, anterior and posterior cingulate cortices, thalamus, and hippocampus; • Reduction of calcitriol in plasma and of DA metabolites in the CSF; • Tolerance.
Experimental animals	• Aggressive behavior; • Increased glial activation in cortical, limbic and nigrostriatal brain areas; • Memory deficits; • Reduction in the levels of hypothalamic, striatal, limbic and cortical 5-HT, 5-HIAA, SERT density and TPH activity; • Reduction in the levels of DA, DAT and TH in the nigrostriatal system.

related factors, such as dose, routes, and regimens of MDMA administration (Baumann et al., 2009; Shokry et al., 2019), but also by factors such as the species (Stone et al., 1987; Logan et al., 1988), age (Reveron et al., 2005; Frau et al., 2016a; Feio-Azevedo et al., 2018; Costa et al., 2019; Chitre et al., 2020), and gender of animals (Pardo-Lozano et al., 2012; Costa et al., 2019), as well as the body temperature (Chen et al., 2020), and the setting of the environment where MDMA is administered (De Win et al., 2004; Piper and Meyer, 2004; Schilt et al., 2010; Price et al., 2014; Shokry et al., 2019).

This review provides a concise overview of recent findings obtained in preclinical and clinical studies that evaluated the noxious effects of MDMA use, with particular regard to neurotoxicity and its underlying mechanisms.

2. Pharmacological effects of MDMA

2.1. Acute effects of MDMA on serotonin (5-HT) and dopamine (DA) release

Studies in experimental animals have demonstrated that the serotonergic and dopaminergic (DAergic) pathways are the main target of MDMA (Green et al., 2003). MDMA can interact, either directly or indirectly, with the 5-hydroxytryptamine (5-HT) transporter (SERT) and the dopamine (DA) transporter (DAT) (Verrico et al., 2007), to stimulate the efflux of 5-HT (Green et al., 2003; Moratalla et al., 2017) and DA from presynaptic vesicles in the caudate-putamen (CPu) and prefrontal cortex (PFC) (Nash, 1990; Baumann et al., 2007); notably, this effect is also displayed by the 3,4-methylenedioxyamphetamine (MDA), the major metabolite of MDMA. Moreover, MDMA and MDA can decrease the activity of tryptophan hydroxylase (TPH) (Schmidt and Taylor, 1987; Stone et al., 1987), an enzyme involved in the synthesis of 5-HT. The possibility that MDMA affects the levels of SERT and the densities of 5-HT receptors in humans is disputed, since the studies performed so far have been mostly characterized by marked methodological heterogeneity (i.e., in the dosages of MDMA used and with regard to the intake of drugs other than MDMA) and by small sample sizes (Müeller et al., 2016). Nevertheless, evidence exists to suggest that that the interaction of MDMA with SERT, and the subsequent release of 5-HT, could be responsible for most of the physical and psychological effects of MDMA in humans (Liechti et al., 2000). This hypothesis may be supported by data obtained in experimental animals that the pharmacological blockade of SERT with fluoxetine (Schmidt, 1989; Malberg et al., 1996; O'Shea et al., 2001), reduces the ability of MDMA to release 5-HT from terminals. Similar findings have been obtained in experimental animals after the blockade of DAT with cocaine, or its analogues, which resulted in a reduced release of DA (Camarero et al., 2002; Peraile et al., 2010). Nevertheless, MDMA appears to act mainly on the serotonergic system in the human brain; accordingly, it is still disputed whether acute consumption of MDMA may affect the DAT in humans (Vizeli and Liechti, 2019).

2.2. Persistent effects of MDMA on 5-HT and DA after repeated exposure or intake of high doses

As mentioned above, controversies exist in the characterization of the long-term central untoward effects elicited by the repeated exposure to MDMA, both in human heavy users and in experimental animals. Despite the large amount of studies that suggest the occurrence of neurotoxic brain damages in experimental animals repeatedly treated with MDMA, some authors have hypothesized that MDMA may be able to down-regulate 5-HT and SERT without causing overt neuronal damage (Kish, 2002; Biezonski and Meyer, 2010; Kivell et al., 2010; Vegting et al., 2016), thus questioning the existence of neurotoxic effects of MDMA. In this regard, it should be considered that many studies in experimental animals generally employ a binge-like dosing regimen, and often do not perform an evaluation of the persistent/delayed serotonergic and/or DAergic modifications induced by MDMA, which may become fully evident long after drug discontinuation (Green et al., 2009).

Regarding the effects of MDMA on the serotonergic system, studies in rats and non-human primates have demonstrated that a single administration of MDMA may reduce the levels of striatal, limbic and cortical 5-HT, an effect that usually recover within 24 h from drug administration, and may decrease the activity of TPH, an effect that usually recovers after longer times (Commins et al., 1987; Lyles and Cadet, 2003). Nevertheless, prolonged and/or repeated exposure to MDMA can cause long-term abnormalities in the serotonergic system of rodents and non-human primates, such as a decrease in the levels of hypothalamic, striatal, limbic and cortical 5-HT and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) (Commins et al., 1987; Schmidt, 1989; Molliver et al., 1990; Cox et al., 2014), reduced SERT density (Biezonski and Meyer, 2010), and a drop in the activity of TPH (De Souza et al., 1990; Molliver et al., 1990). Importantly, these changes have been shown to be persistent, as they could be observed months or even years after drug discontinuation, both in rats (Battaglia et al., 1987; De Souza et al., 1990; Molliver et al., 1990; Cox et al., 2014) and non-human primates (Ricaurte et al., 1988; Ricaurte and McCann, 1992). In this regard, non-human primates have been found to be very sensitive to the noxious effects of MDMA in the brain. Indeed, it has been demonstrated that non-

human primates repeatedly treated with MDMA exhibit depletions of 5-HT and 5-HIAA in different brain regions, such as somatosensory cortex, caudate nucleus, putamen, hippocampus, hypothalamus, and thalamus, as well as in the cerebrospinal fluid (CSF) (Ricaurte et al., 1988; Insel et al., 1989). Moreover, non-human primates repeatedly treated with MDMA have been found to display reduced SERT density when evaluated one year after drug discontinuation by means of positron emission tomography (PET) imaging for [^{11}C]McN-5652, a radioligand that selectively binds to SERT (Scheffel et al., 1998).

Several investigations have also tested the possibility that MDMA can cause serotonergic abnormalities in the human brain. A recent review has summarized the results obtained in imaging studies performed over the last 20 years, concluding that heavy MDMA consumption may be associated with reduced SERT density in several brain regions (i.e., parietal, temporal and occipital lobe, anterior and posterior cingulate cortices, thalamus, and hippocampus) (Müller et al., 2019); nevertheless, it cannot be ruled out that the reduction in SERT density may be reversible, as hypothesized by other authors (Buchert et al., 2006).

Regarding the effects of MDMA on the DAergic system, it is noteworthy that most of the studies available in this respect have been performed in mice. Thus, MDMA elicits a peculiar profile of neurotoxicity in this species that scarcely affects the serotonergic systems while heavily impacting the DAergic nigrostriatal and mesolimbic systems (Colado et al., 2001; Green et al., 2003; Cadet et al., 2007; Granado et al., 2008; Moratalla et al., 2017; Costa et al., 2017, 2021a). Acute MDMA oral administration was found to decrease striatal DA content when administered at 25 °C but not 4 °C (Mueller et al., 2013). Repeated administration of MDMA in mice decreased the levels of both DA and DAT in the CPu (Iravani et al., 2000; Colado et al., 2001; Camarero et al., 2002; Green et al., 2003; Cadet et al., 2007; Costa et al., 2013, 2017; Halpin et al., 2014), as well as the immunoreactivity for tyrosine hydroxylase (TH), the rate-limiting enzyme for DA synthesis, in both the CPu and substantia nigra pars compacta (SNc) (Granado et al., 2008; Moratalla et al., 2017; Costa et al., 2017). The elicitation of DAergic damage by MDMA has long been envisaged as an effect peculiar to mice; however, recent evidence suggests that the repeated administration of MDMA may be toxic for the DAergic system of rats (Cadoni et al., 2017) and non-human primates (Millot et al., 2020) as well. In the study of Cadoni et al. (2017), the exposure of rats to multiple injections of MDMA at adolescence induced DAergic damage that was evident at adulthood and consisted in reduced numbers of TH-positive neurons in both the SNc and ventral tegmental area (VTA), and in decreased immunoreactivity for TH and DAT in the CPu and nucleus accumbens (Cadoni et al., 2017). Millot et al. (2020) first repeatedly treated non-human primates with MDMA then, after a 5-months washout, they administered the same animals with the DAergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is able to induce in experimental animals (i.e., mice and non-human primates) nigrostriatal DAergic degeneration and motor deficits akin to those featuring Parkinson's disease (PD). Interestingly, this study revealed that repeated MDMA administration reduced the availability of DAT in the caudate nucleus and in the putamen, evaluated by means of magnetic resonance imaging techniques that employed the [^{11}C]PE2I (^{11}C-N-(3-iodoprop-2E-enyl)-2beta-carbomethoxy-3beta-(4-methylphenyl)nortropane ligand (Millot et al., 2020). Moreover, and importantly, the same study found that prior exposure to MDMA aggravated MPTP-induced parkinsonism in non-human primates (Millot et al., 2020). These data obtained in non-human primates are of particular relevance, considering their translational potential to the human setting (Harding, 2017; Smith and Galvan, 2018). Interestingly, a similar detrimental interaction between MDMA and the DAergic neurotoxin MPTP has been previously demonstrated by our group in C57BL/6J mice, where the repeated exposure to MDMA during adolescence was found to exacerbate the nigrostriatal, cortical and limbic DAergic damage, as well as glia activation and recognition memory deficits that were induced by MPTP administered at adulthood (Costa et al., 2013, 2014).

The use of illicit psychostimulants has been associated with the presence of abnormal SNc morphology and with increased risk of PD (Tai et al., 2011; Todd et al., 2013). Indeed, a possible link between MDMA use and manifestation of PD has been hypothesized several years ago (Kish, 2003; Deik et al., 2012), based on case-reports of juvenile parkinsonism in MDMA users (Mintzer et al., 1999; O'Suilleabhain and Giller, 2003; Kuniyoshi and Jankovic, 2003). Additionally, McCann et al. (1994) identified a reduction in the DA metabolite homovanillic acid in the CSF of female, but not male, MDMA users, suggestive of possible abnormalities in DAergic systems. Furthermore, metabolomic analysis of plasma samples obtained from individuals who took a single MDMA dose found that calcitriol was decreased after MDMA intake (Boxler et al., 2018). Calcitriol is the active metabolite of vitamin D3, and is able to upregulate trophic factors, including the glial cell line-derived neurotrophic factor, thus eliciting protective effects on DAergic neurons (Cass et al., 2006). Hence, the ability of MDMA to reduce the levels of calcitriol could provide further support to the hypothesis that this amphetamine-related drug may induce DAergic damage in humans. Nevertheless, as of today no convincing evidence exists to unequivocally demonstrate that MDMA use may damage DA neurons and/or terminals in humans (Gerra et al., 2002; Vegting et al., 2016).

Other long-term detrimental effects of MDMA use in humans that are frequently described include cognitive deficits, psychiatric disturbances and the development of tolerance to the pleasurable effects of the drug on the emotional state (Table 2). Recent reviews of the existing literature have suggested that heavy MDMA users are impaired in several cognitive domains, as compared with MDMA-naïve individuals (Garg et al., 2015; Amoroso, 2019). These deficits appear particularly evident for verbal learning, attention, and working memory (Pantoni and Anagnostaras, 2019; Mead and Parrott, 2020), whereas the data available does not consistently demonstrate the existence of detrimental effects of MDMA on other cognitive domains. Moreover, heavy MDMA users frequently complain for the presence of psychiatric disturbances, such as depressed mood, an effect that has been reported to be present up to three weeks after drug intake (Gerra et al., 1998), and anxiety. Finally, regular MDMA users often report diminished responsiveness to the pleasurable effects of the drug and a consequent need for dose escalation, implying the development of tolerance (Mead and Parrott, 2020).

To summarize, most of the experimental evidence suggesting the existence of MDMA-induced neurotoxicity, cognitive and psychiatric dysfunctions and tolerance results from studies performed in heavy recreational users. While it seems plausible that heavy MDMA use may lead to certain neural and behavioral toxic effects, it is important to remark that there is still insufficient evidence to conclude that low to moderate MDMA use is detrimental to human brain structure/function.

3. Mechanisms of neurotoxicity induced by MDMA

Independent investigations have proposed different mechanisms that may be responsible for the neurotoxic effects of MDMA. As of today, the relative importance of the different mechanisms that may underlie the neurotoxicity induced by MDMA is still disputed; moreover, it may be speculated that only the occurrence of synergistic interactions among different mechanisms of toxicity may fully explain the noxious effects elicited by MDMA in the CNS. The available evidence suggests that, among the mechanisms that are most likely to be involved in MDMA-induced neurotoxicity, there are the generation of toxic metabolites of MDMA, the increase in oxidative stress and DA-based quinones, the occurrence of mitochondrial dysfunction, the activation of glial cells and the occurrence of excitotoxic events, the induction of hyperthermia. The available data supporting a role of these mechanisms in MDMA-induced toxicity are summarized in the remainder of this review.

3.1. Role of MDMA toxic metabolites

As local injections of MDMA into various regions of the rodent CNS

fail to recapitulate the neurotoxicity that is observed following the systemic administration of MDMA, several investigations have explored the possibility that MDMA-induced neurotoxicity stems from the generation of toxic metabolites. Metabolism of MDMA may involve a reaction of *O*-demethylation with formation of MDA, which is subsequently converted into 3,4-dihydroxyamphetamine (also known as α-methyldopamine, α-MeDA); alternatively, MDMA can undergo a reaction of *N*-demethylation with formation of 3,4-dihydroxymethamphetamine (HHMA) (Fig. 1). α-MeDA and HHMA can be further metabolized by catechol-*O*-methyltransferase (COMT) to 3-methoxy-4-hydroxyamphetamine (HMA) or to 3-methoxy-4-hydroxymethamphetamine (HMMA), respectively (reviewed by Green et al., 2003) (Fig. 1). α-MeDA is a catecholic compound that can be oxidized to the corresponding ortho-quinone, which is a highly redox-active molecule (Conway et al., 1978), that can be further oxidized leading to the synthesis of reactive oxygen species (ROS) and reactive nitrogen species (RNS), hence it might be involved in the manifestation of MDMA neurotoxicity. Support to this hypothesis is provided by the evidence that the intracerebroventricular injection of α-MeDA into the rat brain produces short-term alterations in the DAergic, serotonergic, and noradrenergic systems (Miller et al., 1996, 1997). However, and interestingly, other studies found that local injections of HHMA-derived metabolites into the rat brain failed to reproduce the neurotoxic effects observed after the peripheral administration of MDMA (Mueller et al., 2011).

As described in detail in the next paragraph, 5-HT may form metabolites able to cause selective 5-HT neuronal damage. Collectively, these data support the hypothesis that the formation of toxic products may be a mechanism that participates in the neurotoxic effects of MDMA, but they also suggest that such a mechanism predominantly involves those metabolites that can be converted into quinones.

3.2. Role of oxidative stress and DA-based quinones

Oxidative stress is defined as an imbalance between the production of ROS and the activity of the antioxidant defense systems. ROS is a collective term that includes not only oxygen radicals (i.e., superoxide and hydroxyl radical), but also certain non-radical species that possess oxidizing properties (i.e., hydrogen peroxide and peroxynitrite). Importantly, the production of ROS can take place not only in the endoplasmic reticulum and mitochondria (Turrens, 2003), but it may also arise from the cytoplasmatic autoxidation of non-radical species that possess a catecholic ring, such as DA.

DA is a chemically stable molecule only when inside the synaptic vesicle. Several studies have corroborated the idea that the effects of MDMA on the release of 5-HT and DA interact to engender neurotoxicity. Thus, DA released by MDMA may enter the 5-HT terminals through the SERT, and then it can be oxidized by monoamine oxidase (MAO) type B enzyme therein, leading to the generation of ROS (Sprague et al., 1998). Moreover, MDMA promotes DA exocytosis and this effect may be exacerbated by hyperthermia (LaVoie and Hastings, 1999), which is also elicited by MDMA (see below), as well as by the co-administration of dopaminomimetic agents such as 3,4-dihydroxy-*l*-phenylalanine (L-DOPA) (Barros-Miñones et al., 2015). The possible involvement of DA release in the neurotoxic effects of MDMA may be explained by the fact that cytosolic DA can be oxidized to reactive quinones, either spontaneously or enzymatically (Stokes et al., 1999). Thus, DA can autoxidize in the presence of metal ions, such as Cu^{2+}, Mn^{2+}, Fe^{3+}, or of copper and ferric chelates; alternatively, DA can be oxidized in physiological conditions by the action of oxidative enzymes, such as xanthine oxidase, peroxidases, lipoxygenases, and copper-containing catechol oxidases (Meiser et al., 2013). We have recently demonstrated that MDMA can activate antioxidant enzymes and that sex differences exist in the antioxidant systems that are triggered in the nigrostriatal pathway in response to the neurotoxic damage induced by MDMA (Costa et al., 2021b). Indeed, we found that nigrostriatal dopaminergic neurons of male mice are more sensitive to the toxic effects mediated by the mitochondrial isoform 2 of superoxide dismutase (SOD2), than to the toxic effects mediated by the cytoplasmatic SOD1 (Costa et al., 2021b). These results are in line with previous findings showing that MDMA administration increases the activity of SOD in mice (Peraile et al., 2013), and that the genetic overexpression of the SOD1 isoform protected mice from the neurotoxic damage induced by MDMA (Jayanthi et al., 1999).

Regarding the mechanisms responsible for the neuronal death elicited by MDMA, since *O*-quinone intermediates are Michael acceptors, it

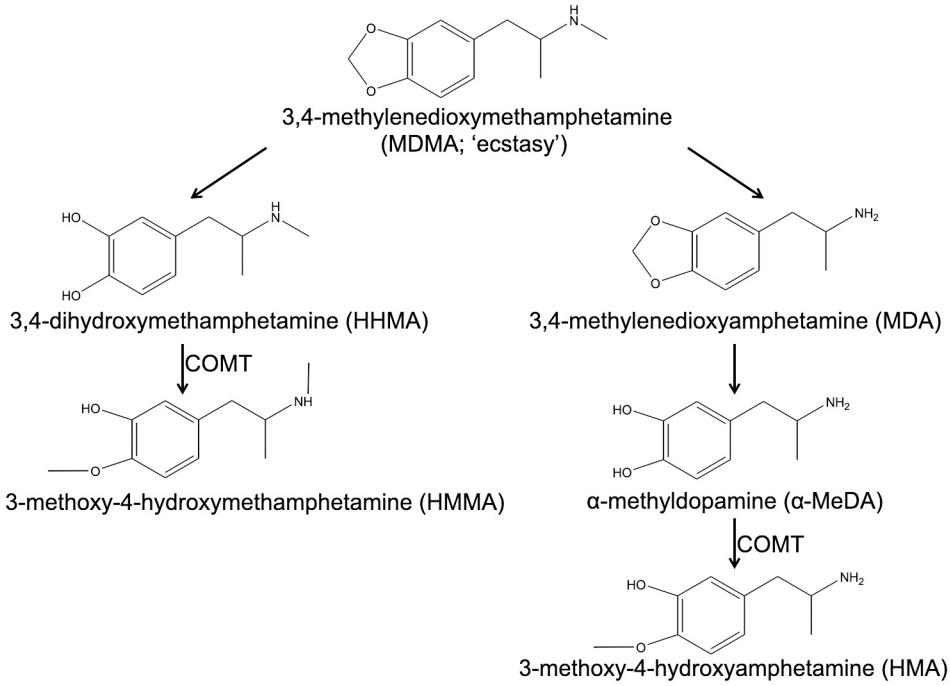

Fig. 1. Bioactivation of 3,4-methylenedioxymethampetamine (MDMA).

is possible that toxicity can occur through alkylation of crucial cellular proteins and/or DNA. It has been also postulated that disfunctions at the level of the NO system can drive neuronal death processes, and dysfunctions in the NO system have been observed in rodents treated with MDMA (Taraska and Finnegan, 1997; Cadet, 1999; Itzhak and Ali, 2006; Moncada and Bolaños, 2006; Costa et al., 2018). Thus, MDMA-administration significantly increase the formation of NO, as well as nitrated proteins bearing the nitrotyrosine modification, in the rat striatum. The nitration of tyrosine particular appears of relevance to MDMA-mediated neurotoxicity, since this aminoacid is essential to the functioning of 5-HT or DA terminals (Quinton and Yamamoto, 2006).

Taken together, metabolic processes that lead to the formation of quinones, ROS, RNS (Halliwell, 1992; Bolton et al., 2000; de Bragança et al., 2017) and/or toxic oxidation products may represent a key factor responsible for the DAergic neurotoxicity exerted by MDMA. For instance, DA quinones can activate microglia (Kuhn et al., 2006), and they can interact with DAT and TH (LaVoie and Hastings, 1999; Whitehead et al., 2001), mitochondrial complexes I and IV (Berman and Hastings, 1999), and proteasome (Zafar et al., 2006), with potential deleterious effects for neuronal homeostasis (Fig. 2). Thus, activated microglia releases pro-inflammatory mediators, as detailed below. Moreover, DA quinones covalently bind to TH and DAT, thus inhibiting the function of these proteins, and they can also disrupt mitochondrial respiration in the brain (Berman and Hastings, 1999). Furthermore, inhibition of proteasome may lead to the accumulation of misfolded proteins and their aggregation, causing DAergic cell demise (Zhou and Lim, 2009).

In addition to DA autoxidation, the increase in oxidative stress elicited by MDMA may also stem from the oxidation of 5-HT (Fig. 3). Thus, as mentioned above, MDMA releases 5-HT which may be oxidized to hydrogen peroxide, an effect resulting in the subsequent formation of hydroxyl radical (Heuther et al., 1990). Besides, 5-HT may be oxidized to form metabolites that are thought to cause selective 5-HT neuronal damage, such as 5,7-dihydroxytryptamine (5,7-DHT). Indeed, the intracerebroventricular administration of 5,7-DHT in the PFC of rats has been found to deplete 5-HT and SERT and to persistently increase the levels of markers of astrocyte activation, such as heat shock protein 32 (Wang et al., 2005) and glial fibrillary acidic protein (GFAP) (Wang et al., 2005; Baumann et al., 2007). Interestingly, it has been demonstrated that reactive astrocytes, which are activated as a consequence of the release of pro-inflammatory mediators from activated microglia, lose the ability to promote neuronal survival, outgrowth, synaptogenesis and phagocytosis, being instead able to promote the death of neurons and oligodendrocytes (Liddelow et al., 2017).

It is now well accepted that an increase in the levels of ROS contributes to a number of pathological processes in the CNS such as, for example, aging (Stefanatos and Sanz, 2018), apoptosis (Redza-Dutordoir and Averill-Bates, 2016), and cellular injury during ischemia and reperfusion (Yang et al., 2019). Since 1990s, several lines of preclinical evidence have accumulated to support the possibility that the formation of ROS is among the mechanisms that sustain MDMA-induced DAergic and serotonergic neurotoxicity. Thus, data obtained in rats have shown that repeated MDMA administration increases the formation of hydroxyl radicals (Colado et al., 1997, 1999a, 1999b; Shankaran et al., 1999a, 1999b). Interestingly, Shankaran et al. (1999a) demonstrated that the ability of repeated MDMA administration to increase the generation of hydroxyl radicals in the CPu of rats paralleled the rise in the extracellular concentration of striatal DA (Shankaran et al., 1999a), which may undergo oxidation, as previously explained. Interestingly, the same group evaluated, after repeated MDMA, the extracellular concentration of striatal 2,3-dihydroxybenzoic acid, as an index of hydroxyl radical generation. Results obtained showed that MDMA increased the levels of 2,3-dihydroxybenzoic acid, which were reduced by treatment with fluoxetine, that attenuated also the striatal 5-HT depletion (Shankaran et al., 1999b). Finally, it has been widely demonstrated that certain psychoactive substances, when administered together with MDMA, may enhance the pro-oxidant effects of this amphetamine-related drug and also lead to an exacerbation of neurotoxicity. Hence, the co-administration of caffeine has been found to potentiate the release of DA induced by MDMA in the mouse CPu (Górska et al., 2018); given the auto-oxidizing properties of DA, this effect could underlie the increase in DAergic damage that has been observed in the mouse nigrostriatal system after the combined administration of caffeine and MDMA, compared with MDMA alone (Frau et al., 2016b). Conversely, the

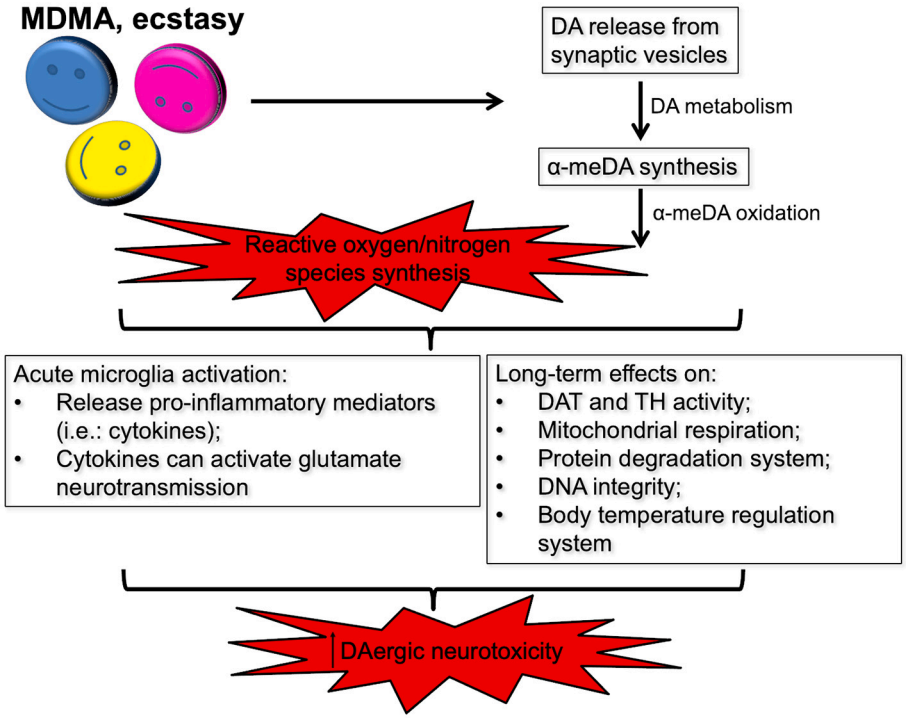

Fig. 2. Consequences of oxidative events induced by MDMA administration in the DAergic nerve terminal.

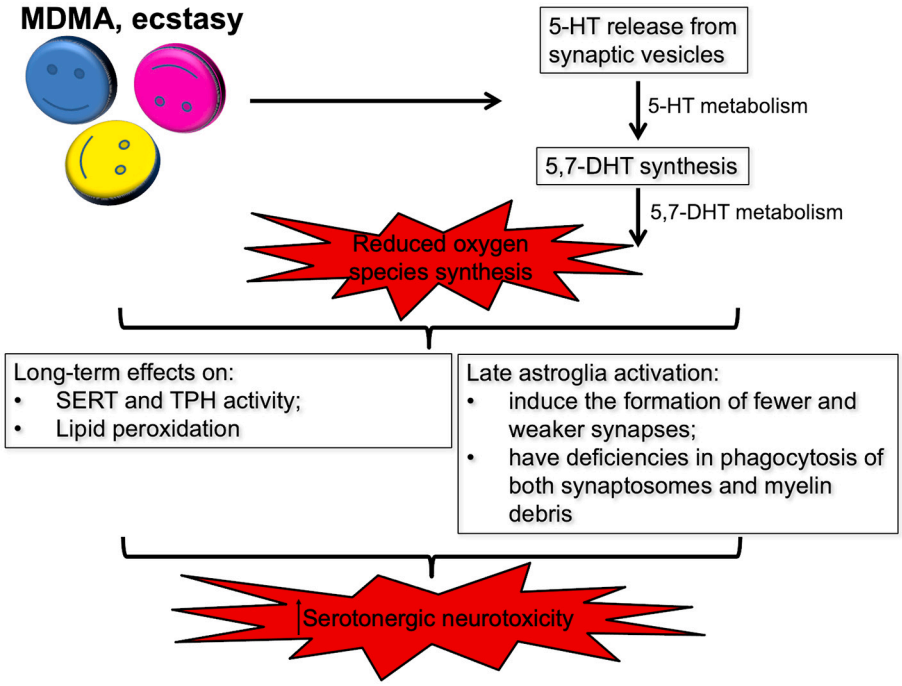

Fig. 3. Consequences of oxidative events induced by MDMA administration in the serotonergic nerve terminal.

combined administration of antioxidant molecules (Mehdizadeh et al., 2012; Shih et al., 2016), as well as of free radical scavengers (Colado et al., 1999a), has been found to curb the neurotoxic effects that MDMA elicits in the serotonergic system of rats.

Preclinical studies have demonstrated that the pro-oxidant effects of MDMA may also result in increased lipid peroxidation in serotonergic nerve terminals, which has been demonstrated in the PFC, hippocampus and CPu of experimental rodents repeatedly treated with MDMA (Sprague and Nichols, 1995; Alves et al., 2009; Peraile et al., 2013; Budzynska et al., 2018). Moreover, repeated treatment with MDMA has been found also to affect the integrity of DNA in the hippocampus (Frenzilli et al., 2007) and nigrostriatal system (Fornai et al., 2004) of mice. The detrimental effects of MDMA on DNA integrity have been manifested as single- and double–strand breaks and, interestingly, they have been found to persist even after the exhaustion of oxidative stress vanished, as shown in the mouse hippocampus by Frenzilli et al. (2007) and also by our group in the mouse PFC (Górska et al., 2018) and in the rat PFC and hippocampus (Fig. 4).

Taken together, the preclinical evidence available would strongly support the possibility that increased oxidative stress participates in the neurotoxic effects of MDMA. Nevertheless, to our knowledge, the ability of MDMA to induce oxidative events in humans has been demonstrated in plasma samples only (Zhou et al., 2003), which claims for further investigations aimed at clarifying whether MDMA may increase the levels of oxidative stress in the CNS of humans and whether such an effect may eventually lead to neuronal damage.

3.3. Role of mitochondrial dysfunction

Repeated MDMA administration has been demonstrated to inhibit

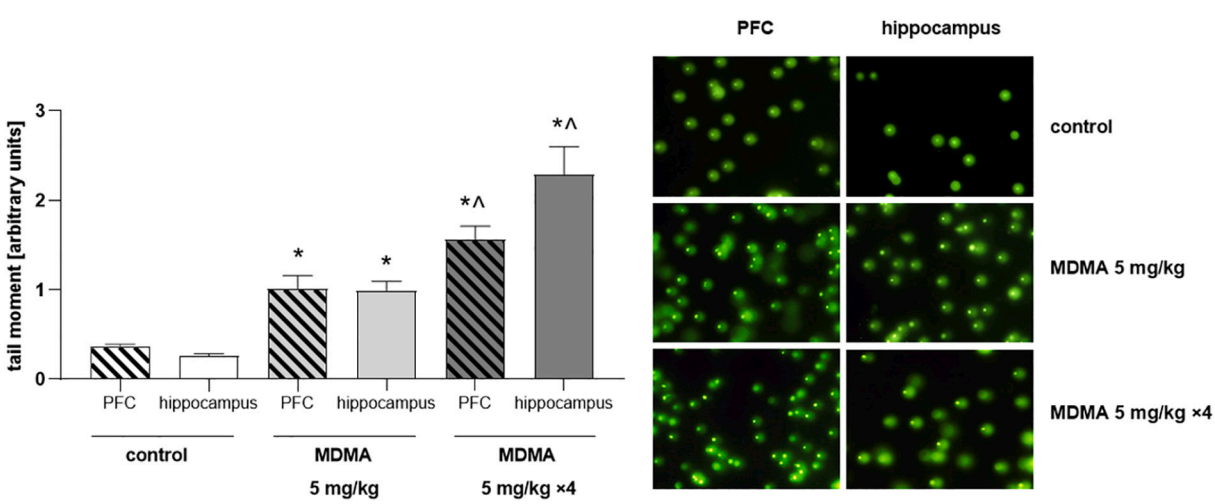

Fig. 4. Effect of acute MDMA (5 mg/kg i.p.) or repeated MDMA (4 × 5 mg/kg, i.p., every second day for 8 days) on DNA integrity in the rat prefrontal cortex (PFC) and hippocampus evaluated with the comet assay technique. Data represent a tail moment defined as the product of tail length and the fraction of total DNA in the tail. Tail moment incorporates a measure of both the smallest detectable size of migrating DNA (reflected in the comet tail length) and the number of damaged pieces (represented by the intensity of DNA in the tail). * significant difference vs. control; ^ significant difference between acute and repeated treatment.

the complex I of the electron transport chain (Puerta et al., 2010), thus impairing the mitochondrial respiration and eventually leading to an increased production of ROS. Building on this evidence, several studies have investigated neuroprotective strategies that may prevent the mitochondrial damage induced by MDMA. In this regard, it has been shown that the inhibition of DA metabolism with pargyline, a MAO inhibitor, afforded a significant protection against the inhibition of complex I induced by MDMA and the associated neurotoxicity (Barros-Miñones et al., 2015). Moreover, another study found that physical exercise attenuated the increase in ROS production induced by a single administration of MDMA in mitochondria isolated from rat brain, along with mitochondria swelling, damage of the mitochondrial outer membrane damage, and amount of cytochrome *c* released from mitochondria (Taghizadeh et al., 2016). Although preclinical studies suggest that mitochondrial dysfunction may play an important role in the neurotoxic effects of MDMA, the importance of this mechanism in the toxic effects of MDMA in humans is ill defined and further studies in this respect are warranted.

3.4. Role of glial cells and excitotoxic events

In the CNS, glial cells, and particularly microglial cells and astrocytes, play a key protective and supporting role for neurons (Fetler and Amigorena, 2005; Bélanger and Magistretti, 2009).

In physiological conditions, microglial cells exist in a resting state, but in response to an insult they immediately increase in size, migrate to the site of injury and release pro-inflammatory mediators, such as cytokines. In the long term, microglia activation is a neuroprotective event, as it results in the phagocytosis of dying and dead cells (Kreutzberg, 1996; Badoer, 2010; Kettenmann et al., 2013). A rapid activation of microglia paired with an increase in IL-1β concentrations has been demonstrated in the cortex and hypothalamus of rats after a single MDMA administration (Orio et al., 2004). Moreover, subsequent studies have confirmed the ability of MDMA to rapidly activate microglia, by demonstrating that experimental rodents repeatedly treated with MDMA displayed increased microglia activation in cortical and limbic brain areas (Costa et al., 2014; Herndon et al., 2014a). A crucial point in understanding the interplay between the microglia activation and the neurotoxicity elicited by MDMA is the fact that the cytokines that are produced during microglia activation can activate glutamate neurotransmission, and in turn promote excitotoxicity (Zou and Crews, 2005). In this regard, it is noteworthy that a previous study in rats has demonstrated that microglia activation observed in the hippocampus after repeated exposure to MDMA correlated with the increase in extracellular glutamate in the same region (Anneken and Gudelsky, 2012).

Astrocytes are other major glia cells that are involved in the reuptake of glutamate released at the synaptic level, thus optimizing neuronal function and preventing glutamate excitotoxicity (Anderson and Swanson, 2000). Astrocytes are a second line of defense for the CNS and, similar to microglia, they undergo morphological and functional changes in response to insults (Sofroniew and Vinters, 2010; Scuderi et al., 2013; Rossi, 2015). Reactive astrocytes are characterized by hypertrophy of their cellular processes and altered expression of many genes including the up-regulation of GFAP (Liddelow and Barres, 2017). Interestingly, Thomas et al. (2004) found that not only MDMA was able to increase microglia activation in the mouse brain, but also that MDMA increased GFAP staining. As explained above, reactive astroglia may contribute to neuronal death (Liddelow et al., 2017), corroborating the idea of a link between glial cells activation and neurotoxicity. The study of Thomas et al. (2004) also found that amphetamine-related drugs that are devoid of neurotoxic effects, such as l-methamphetamine, fenfluramine, and 2,5-dimethoxy-4-iodoamphetamine, failed to increase GFAP staining and to induce microglial activation in mice (Thomas et al., 2004). Thus, these findings further indicate that both microglia and astroglia are critically involved in the neurotoxic effects of MDMA.

We and others have demonstrated that in mice the repeated administration of MDMA induces activation of glia cells in the nigrostriatal DAergic system that may be associated with neurotoxicity (Granado et al., 2008; Khairnar et al., 2010; Ruiz-Medina et al., 2011; Costa et al., 2013; Frau et al., 2013, 2016a, 2016b). Crucial factors in MDMA-induced glia activation and associated neurotoxicity appear to be age and gender of the animals, as well as the environmental setting (i.e., crowding and temperature) where MDMA is administered (Lopez-Rodriguez et al., 2014; Frau et al., 2016a; Costa et al., 2019). Moreover, other studies have shown that glia activation and neurotoxicity induced by MDMA in the DAergic nigrostriatal system may be influenced by drugs that are given concomitantly with MDMA. For example, experimental rodents treated with MDMA in combination with caffeine or Δ^9-tetrahydrocannabinol have been shown to display an exacerbation of proinflammatory and neurotoxic effects in the DAergic nigrostriatal tract, compared with MDMA alone (Khairnar et al., 2010; Lopez-Rodriguez et al., 2014; Frau et al., 2016b; Górska et al., 2018).

Understanding the role of glia in the noxious central effects of MDMA in humans is difficult, since only few studies exist that examined the modifications involving the immune response to MDMA administration in humans (Pacifici et al., 1999, 2001a, 2001b, 2002).

3.5. Role of hyperthermia

As reported in Table 1, the induction of hyperthermia is a common acute untoward effect of MDMA administration in experimental animals and humans. A major factor that influences the manifestation of hyperthermia is the dose of MDMA: low/moderate doses have little effect, whereas high or repeated doses cause a marked increase in body temperature that can degenerate in hyperpyrexia, which may be lethal (Parrott, 2012; Jahns et al., 2018). Among the other factors that should be taken into account when considering the hyperthermic effects of MDMA is the environmental setting where the drug is experienced. Indeed, it has been suggested that social crowding, as the one occurring in dance clubs, house parties and bars (Bedi and Redman, 2006), together with loud music (Gesi et al., 2004), may act as factors that promote the manifestation of hyperthermia, although the impact of these factors on body temperature are harder to investigate in human users, compared with experimental animals. Nevertheless, independent preclinical studies in experimental rodents have demonstrated that neurotoxic and/or neuroinflammatory effects of MDMA may be exacerbated by hyperthermia, as well as by drug experience in setting that involve environmental high temperatures and/or cage crowding (Goñi-Allo et al., 2008; Takamatsu et al., 2011; Frau et al., 2013, 2016a). In line with these findings are the results of other preclinical studies that involved the administration of MDMA at low ambient temperatures (Goñi-Allo et al., 2008; Adori et al., 2011), or the co-administration of MDMA with compounds able to lower the body temperature (Touriño et al., 2010), that found a reduced neurotoxicity of MDMA in these experimental conditions, as compared with standard experimental settings. A review recently published by Vercoulen and Hondebrink (2021) analyzed the literature from in vitro, animal and human studies concerned with the co-administration of MDMA and alcohol, revealing that alcohol seems to decrease body temperature, although the influence of this effect on MDMA pharmacokinetics appears unclear (Vercoulen and Hondebrink, 2021). Interestingly, evidence exists to suggest that the COMT enzymes may be an important player in the regulation of the hyperthermic response to MDMA and, in turn, of the neurotoxic effects of this amphetamine-related drug. In fact, the pharmacological inhibition of COMT in the rat has been found to potentiate the serotonergic deficits induced by MDMA and to exacerbate the hyperthermic response to MDMA (Herndon et al., 2014b).

4. Conclusions

Preclinical studies indicate that MDMA may damage neuronal bodies

and terminals. In this review, we have presented evidence that the generation of toxic metabolites of MDMA, the increase in oxidative stress and DA-based quinones, the occurrence of mitochondrial dysfunction, the activation of glial cells and the occurrence of excitotoxic events, the induction of hyperthermia are involved in causing the neurodegenerative effects of MDMA in experimental animals. Although there is a growing consensus that MDMA can cause neurotoxic effects also in humans, no conclusive evidence of long-lasting serotonergic and/or DAergic neurotoxic damage has been obtained so far in human MDMA users. Nevertheless, the use of MDMA poses a serious health concern, since it may induce alterations in brain functions that may persist even after drug discontinuation. Thus, investigation of the behavioral outcome of the neurotoxic insults caused by MDMA in experimental animals is essential for better understanding the consequences of human abuse of this amphetamine-related drug.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this review.

Acknowledgements

Funding: Dr. Giulia Costa gratefully acknowledges the financial support from PRIN 2015 (Pr. 2015R9ASHT, PI Prof. Micaela Morelli) and PON AIM (PON RICERCA E INNOVAZIONE 2014-2020, - AZIONE I.2. D.D. N.407 DEL 27 FEBBRAIO 2018 - "ATTRACTION AND INTERNATIONAL MOBILITY").

References

Adori, C., Andó, R.D., Balázsa, T., Soti, C., Vas, S., Palkovits, M., Kovács, G.G., Bagdy, G., 2011. Low ambient temperature reveals distinct mechanisms for MDMA-induced serotonergic toxicity and astroglial Hsp27 heat shock response in rat brain. Neurochem. Int. 59, 695–705. https://doi.org/10.1016/j.neuint.2011.06.017.

Aguilar, M.A., García-Pardo, M.P., Parrott, A.C., 2020. Of mice and men on MDMA: a translational comparison of the neuropsychobiological effects of 3,4-methylenedioxymethamphetamine ('Ecstasy'). Brain Res. 1727, 146556. https://doi.org/10.1016/j.brainres.2019.146556.

Alves, E., Binienda, Z., Carvalho, F., Alves, C.J., Fernandes, E., de Lourdes, B.M., Tavares, M.A., Summavielle, T., 2009. Acetyl-L-carnitine provides effective in vivo neuroprotection over 3,4-methylenedioximethamphetamine-induced mitochondrial neurotoxicity in the adolescent rat brain. Neuroscience. 158, 514–523. https://doi.org/10.1016/j.neuroscience.2008.10.041.

Amoroso, T., 2019. The spurious relationship between ecstasy use and neuro cognitive deficits: a Bradford Hill review. Int. J. Drug Policy 64, 47–53.

Anderson, C.M., Swanson, R.A., 2000. Astrocyte glutamate transport: review of properties, regulation, and physiological functions. Glia. 32, 1–14.

Anneken, J.H., Gudelsky, G.A., 2012. MDMA produces a delayed and sustained increase in the extracellular concentration of glutamate in the rat hippocampus. Neuropharmacology. 63, 1022–1027. https://doi.org/10.1016/j.neuropharm.2012.07.026.

Badoer, E., 2010. Microglia: activation in acute and chronic inflammatory states and in response to cardiovascular dysfunction. Int. J. Biochem. Cell Biol. 42, 1580–1585. https://doi.org/10.1016/j.biocel.2010.07.005.

Barros-Miñones, L., Goñi-Allo, B., Suquia, V., Beitia, G., Aguirre, N., Puerta, E., 2015. Contribution of dopamine to mitochondrial complex I inhibition and dopaminergic deficits caused by methylenedioxymethamphetamine in mice. Neuropharmacology. 93, 124–133. https://doi.org/10.1016/j.neuropharm.2015.01.025.

Battaglia, G., Yeh, S.Y., O'Hearn, E., Molliver, M.E., Kuhar, M.J., De Souza, E.B., 1987. 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [3H]paroxetine-labeled serotonin uptake sites. J. Pharmacol. Exp. Ther. 242, 911–916.

Baumann, M.H., Wang, X., Rothman, R.B., 2007 Jan. 3,4-Methylenedioxymethamphetamine (MDMA) neurotoxicity in rats: a reappraisal of past and present findings. Psychopharmacology (Berl) 189 (4), 407–424. https://doi.org/10.1007/s00213-006-0322-6.

Baumann, M.H., Zolkowska, D., Kim, I., Scheidweiler, K.B., Rothman, R.B., Huestis, M.A., 2009. Effects of dose and route of administration on pharmacokinetics of (+ or -)-3,4-methylenedioxymethamphetamine in the rat. Drug Metab. Dispos. 37, 2163–2170. https://doi.org/10.1124/dmd.109.028506.

Bedi, G., Redman, J., 2006. Recreational ecstasy use: acute effects potentiated by ambient conditions? Neuropsychobiology. 53, 113 author reply 114. https://doi.org/10.1159/000092220.

Bélanger, M., Magistretti, P.J., 2009. The role of astroglia in neuroprotection. Dial. Clin. Neurosci. 11, 281–295. https://doi.org/10.31887/DCNS.2009.11.3/mbelanger.

Berman, S.B., Hastings, T.G., 1999. Dopamine oxidation alters mitochondrial respiration and induces permeability transition in brain mitochondria: implications for Parkinson's disease. J. Neurochem. 73, 1127–1137. https://doi.org/10.1046/j.1471-4159.1999.0731127.x.

Biezonski, D.K., Meyer, J.S., 2010. Effects of 3,4-methylenedioxymethamphetamine (MDMA) on serotonin transporter and vesicular monoamine transporter 2 protein and gene expression in rats: implications for MDMA neurotoxicity. J. Neurochem. 112, 951–962. https://doi.org/10.1111/j.1471-4159.2009.06515.x.

Bolton, J.L., Trush, M.A., Penning, T.M., Dryhurst, G., Monks, T.J., 2000. Role of quinones in toxicology. Chem. Res. Toxicol. 13, 135–160. https://doi.org/10.1021/tx9902082.

Boxler, M.I., Streun, G.L., Liechti, M.E., Schmid, Y., Kraemer, T., Steuer, A.E., 2018. Human metabolome changes after a single dose of 3,4-methylenedioxymethamphetamine (MDMA) with special focus on steroid metabolism and inflammation processes. J. Proteome Res. 17, 2900–2907.

Buchert, R., Thomasius, R., Petersen, K., Wilke, F., Obrocki, J., Nebeling, B., Wartberg, L., Zapletalova, P., Clausen, M., 2006. Reversibility of ecstasy-induced reduction in serotonin transporter availability in polydrug ecstasy users. Eur. J. Nucl. Med. Mol. Imaging 33, 188–199.

Budzynska, B., Wnorowski, A., Kaszubska, K., Biala, G., Kruk-Słomka, M., Kurzepa, J., Boguszewska-Czubara, A., 2018. Acute MDMA and nicotine co-administration: behavioral effects and oxidative stress processes in mice. Front. Behav. Neurosci. 12, 149. https://doi.org/10.3389/fnbeh.2018.00149.

Cadet, J.L., 1999. Neurotoxicity of drugs of abuse. In: Koliatsos Vassilis, E. (Ed.), Cell Death and Diseases of the Nervous System. Humana Press, pp. 521–526. ISBN 978-1-4612-1602-5.

Cadet, J.L., Krasnova, I.N., Jayanthi, S., Lyles, J., 2007. Neurotoxicity of substituted amphetamines: molecular and cellular mechanisms. Neurotox. Res. 11, 183–202. https://doi.org/10.1007/BF03033567.

Cadoni, C., Pisanu, A., Simola, N., Frau, L., Porceddu, P.F., Corongiu, S., Dessì, C., Sil, A., Plumitallo, A., Wardas, J., Di Chiara, G., 2017. Widespread reduction of dopamine cell bodies and terminals in adult rats exposed to a low dose regimen of MDMA during adolescence. Neuropharmacology 123, 385–394.

Camarero, J., Sanchez, V., O'Shea, E., Green, A.R., Colado, M.I., 2002. Studies, using in vivo microdialysis, on the effect of the dopamine uptake inhibitor GBR 12909 on 3,4-methylenedioxymethamphetamine ('ecstasy')-induced dopamine release and free radical formation in the mouse striatum. J. Neurochem. 81, 961–972. https://doi.org/10.1046/j.1471-4159.2002.00879.x.

Cass, W.A., Smith, M.P., Peters, L.E., 2006. Calcitriol protects against the dopamine- and serotonin-depleting effects of neurotoxic doses of methamphetamine. Ann. N. Y. Acad. Sci. 1074, 261–271.

Chen, Y., Tran, H.T.N., Saber, Y.H., Hall, F.S., 2020. High ambient temperature increases the toxicity and lethality of 3,4-methylenedioxymethamphetamine and methcathinone. Pharmacol. Biochem. Behav. 192, 172912. https://doi.org/10.1016/j.pbb.2020.172912.

Chitre, N.M., Bagwell, M.S., Murnane, K.S., 2020. The acute toxic and neurotoxic effects of 3,4-methylenedioxymethamphetamine are more pronounced in adolescent than adult mice. Behav. Brain Res. 380, 112413. https://doi.org/10.1016/j.bbr.2019.112413.

Colado, M.I., O'Shea, E., Granados, R., Murray, T.K., Green, A.R., 1997. In vivo evidence for free radical involvement in the degeneration of rat brain 5-HT following administration of MDMA (ecstasy) and pchloroamphetamine but not the degeneration following fenfluramine. Br. J. Pharmacol. 121, 889–900.

Colado, M.I., O'Shea, E., Estaban, B., Granados, R., Green, A.R., 1999a. In vivo evidence against clomethiazole being neuroprotective against MDMA ("ecstasy")-induced degeneration of rat brain 5-HT terminals by a free radical scavenging mechanism. Neuropharmacology 38, 307–314.

Colado, M.I., O'Shea, E., Granados, R., Estaban, B., Martin, A.B., Green, A.R., 1999b. Studies on the role of dopamine in the degeneration of 5-HT nerve endings in the brain of dark Agouti rats following 3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy') administration. Br. J. Pharmacol. 126, 911–924.

Colado, M.I., Camarero, J., Mechan, A.O., Sanchez, V., Esteban, B., Elliott, J.M., Green, A.R., 2001. A study of the mechanisms involved in the neurotoxic action of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') on dopamine neurones in mouse brain. Br. J. Pharmacol. 134, 1711–1723. https://doi.org/10.1038/sj.bjp.0704435.

Commins, D.L., Vosmer, G., Virus, R.M., Woolverton, W.L., Schuster, C.R., Seiden, L.S., 1987. Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain. J. Pharmacol. Exp. Ther. 241, 338–345.

Conway, E.L., Louis, W.J., Jarrott, B., 1978. Acute and chronic administration of alpha-methyldopa: regional levels of endogenous and alpha-methylated catecholamines in rat brain. Eur. J. Pharmacol. 52, 271–280. https://doi.org/10.1016/0014-2999(78)90279-0.

Costa, G., Frau, L., Wardas, J., Pinna, A., Plumitallo, A., Morelli, M., 2013. MPTP-induced dopamine neuron degeneration and glia activation is potentiated in MDMA-pretreated mice. Mov. Disord. 28, 1957–1965.

Costa, G., Simola, N., Morelli, M., 2014. MDMA administration during adolescence exacerbates MPTP-induced cognitive impairment and neuroinflammation in the hippocampus and prefrontal cortex. Psychopharmacology 231, 4007–4018.

Costa, G., Morelli, M., Simola, N., 2017. Progression and persistence of neurotoxicity induced by MDMA in dopaminergic regions of the mouse brain and association with noradrenergic, GABAergic, and serotonergic damage. Neurotox. Res. 32, 563–574.

Costa, G., Morelli, M., Simola, N., 2018. Repeated administration of 3,4-methylenedioxymethamphetamine (MDMA) elevates the levels of neuronal nitric oxide synthase in the nigrostriatal system: possible relevance to neurotoxicity. Neurotox. Res. 34, 763–768. https://doi.org/10.1007/s12640-018-9892-4.

Costa, G., Porceddu, P.F., Serra, M., Casu, M.A., Schiano, V., Napolitano, F., Pinna, A., Usiello, A., Morelli, M., 2019. Lack of Rhes increases MDMA-induced neuroinflammation and dopamine neuron degeneration: role of gender and age. Int. J. Mol. Sci. 20, 1556.

Costa, G., De Luca, M.A., Piras, G., Marongiu, J., Fattore, L., Simola, N., 2020 May. Neuronal and peripheral damages induced by synthetic psychoactive substances: an update of recent findings from human and animal studies. Neural Regen Res 15 (5), 802–816. https://doi.org/10.4103/1673-5374.268895.

Costa, G., Spulber, S., Paci, E., Casu, M.A., Ceccatelli, S., Simola, N., Morelli, M., 2021a. In utero exposure to dexamethasone causes a persistent and age-dependent exacerbation of the neurotoxic effects and glia activation induced by MDMA in dopaminergic brain regions of C57BL/6J mice. Neurotoxicology. 83, 1–13. https://doi.org/10.1016/j.neuro.2020.12.005.

Costa, G., Caputi, F.F., Serra, M., Simola, N., Rullo, L., Stamatakos, S., Sanna, F., Germain, M., Martinoli, M.G., Candeletti, S., Morelli, M., Romualdi, P., 2021b. Activation of antioxidant and proteolytic pathways in the nigrostriatal dopaminergic system after 3,4-methylenedioxymethamphetamine administration: sex-related differences. Front. Pharmacol. 12, 713486. https://doi.org/10.3389/fphar.2021.713486.

Cox, B.M., Shah, M.M., Cichon, T., Tancer, M.E., Galloway, M.P., Thomas, D.M., Perrine, S.A., 2014. Behavioral and neurochemical effects of repeated MDMA administration during late adolescence in the rat. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 48, 229–235. https://doi.org/10.1016/j.pnpbp.2013.09.021.

de Bragança, A.C., Moreau, R.L.M., de Brito, T., Shimizu, M.H.M., Canale, D., de Jesus, D. A., Silva, A.M.G., Gois, P.H., Seguro, A.C., Magaldi, A.J., 2017. Ecstasy induces reactive oxygen species, kidney water absorption and rhabdomyolysis in normal rats. Effect of N-acetylcysteine and allopurinol in oxidative stress and muscle fiber damage. PLoS One 12, e0179199.

De Souza, E.B., Battaglia, G., Insel, T.R., 1990. Neurotoxic effect of MDMA on brain serotonin neurons: evidence from neurochemical and radioligand binding studies. Ann. N. Y. Acad. Sci. 600, 682–697 discussion 697-698. https://doi.org/10.1111/j.1749-6632.1990.tb16918.x.

de Win, M.M., Reneman, L., Reitsma, J.B., den Heeten, G.J., Booij, J., van den Brink, W., 2004. Mood disorders and serotonin transporter density in ecstasy users–the influence of long-term abstention, dose, and gender. Psychopharmacology 173, 376–382. https://doi.org/10.1007/s00213-003-1723-4.

Deik, A., Saunders-Pullman, R., Luciano, M.S., 2012. Substance of abuse and movement disorders: complex interactions and comorbidities. Curr. Drug Abuse Rev. 5, 243–253. https://doi.org/10.2174/1874473711205030243.

European Drug Report, 2017. Trends and Developments-EMCDDA, Lisbon, June 2017.

Feio-Azevedo, R., Costa, V.M., Barbosa, D.J., Teixeira-Gomes, A., Pita, I., Gomes, S., Pereira, F.C., Duarte-Araújo, M., Duarte, J.A., Marques, F., Fernandes, E., Bastos, M. L., Carvalho, F., Capela, J.P., 2018. Aged rats are more vulnerable than adolescents to "ecstasy"-induced toxicity. Arch. Toxicol. 92, 2275–2295.

Fetler, L., Amigorena, S., 2005. Neuroscience. Brain under surveillance: the microglia patrol. Science. 309, 392–393. https://doi.org/10.1126/science.1114852.

Fornai, F., Lenzi, P., Frenzilli, G., Gesi, M., Ferrucci, M., Lazzeri, G., Biagioni, F., Nigro, M., Falleni, A., Giusiani, M., Pellegrini, A., Blandini, F., Ruggieri, S., Paparelli, A., 2004. DNA damage and ubiquitinated neuronal inclusions in the substantia nigra and striatum of mice following MDMA (ecstasy). Psychopharmacology 173, 353–363. https://doi.org/10.1007/s00213-003-1708-3.

Frau, L., Simola, N., Plumitallo, A., Morelli, M., 2013. Microglial and astroglial activation by 3,4- methylenedioxymethamphetamine (MDMA) in mice depends on S(+) enantiomer and is associated with an increase in body temperature and motility. J. Neurochem. 124, 69–78.

Frau, L., Simola, N., Porceddu, P.F., Morelli, M., 2016a. Effect of crowding, temperature and age on glia activation and dopaminergic neurotoxicity induced by MDMA in the mouse brain. Neurotoxicology 56, 127–138.

Frau, L., Costa, G., Porceddu, P.F., Khairnar, A., Castelli, M.P., Ennas, M.G., Madeddu, C., Wardas, J., Morelli, M., 2016b. Influence of caffeine on 3,4-methylenedioxymethamphetamine-induced dopaminergic neuron degeneration and neuroinflammation is age-dependent. J. Neurochem. 136, 148–162.

Frenzilli, G., Ferrucci, M., Giorgi, F.S., Blandini, F., Nigro, M., Ruggieri, S., Murri, L., Paparelli, A., Fornai, F., 2007. DNA fragmentation and oxidative stress in the hippocampal formation: a bridge between 3,4-methylenedioxymethamphetamine (ecstasy) intake and long-lasting behavioral alterations. Behav. Pharmacol. 18, 471–481. https://doi.org/10.1097/FBP.0b013e3282d518aa.

Garg, A., Kapoor, S., Goel, M., Chopra, S., Chopra, M., Kapoor, A., McCann, U.D., Behera, C., 2015. Functional magnetic resonance imaging in abstinent MDMA users: a review. Curr. Drug Abuse Rev. 8, 15–25.

Gerra, G., Zaimovic, A., Giucastro, G., Maestri, D., Monica, C., Sartori, R., Caccavari, R., Delsignore, R., 1998. Serotonergic function after (+/−)3,4-methylenedioxymethamphetamine ('Ecstasy') in humans. Int. Clin. Psychopharmacol. 13, 1–9. https://doi.org/10.1097/00004850-199801000-00001.

Gerra, G., Zaimovic, A., Moi, G., Giusti, F., Gardini, S., Delsignore, R., Laviola, G., Macchia, T., Brambilla, F., 2002. Effects of (+/−) 3,4-methylenedioxymethamphetamine(ecstasy) on dopamine system function in humans. Behav. Brain Res. 134, 403–410.

Gesi, M., Ferrucci, M., Giusiani, M., Lenzi, P., Lazzeri, G., Alessandrì, M.G., Salvadorini, A., Fulceri, F., Pellegrini, A., Fornai, F., Paparelli, A., 2004. Loud noise enhances nigrostriatal dopamine toxicity induced by MDMA in mice. Microsc. Res. Tech. 64, 297–303. https://doi.org/10.1002/jemt.20084.

Goñi-Allo, B.O., Mathúna, B., Segura, M., Puerta, E., Lasheras, B., de la Torre, R., Aguirre, N., 2008. The relationship between core body temperature and 3,4-methylenedioxymethamphetamine metabolism in rats: implications for neurotoxicity. Psychopharmacology 197, 263–278. https://doi.org/10.1007/s00213-007-1027-1.

Górska, A.M., Kamińska, K., Wawrzczak-Bargieła, A., Costa, G., Morelli, M., Przewłocki, R., Kreiner, G., Gołembiowska, K., 2018. Neurochemical and neurotoxic effects of MDMA (ecstasy) and caffeine after chronic combined administration in mice. Neurotox. Res. 33, 532–548.

Granado, N., O'Shea, E., Bove, J., Vila, M., Colado, M.I., Moratalla, R., 2008. Persistent MDMA-induced dopaminergic neurotoxicity in the striatum and substantia nigra of mice. J. Neurochem. 107, 1102–1112.

Green, A.R., Mechan, A.O., Elliott, J.M., O'Shea, E., Colado, M.I., 2003. The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). Pharmacol. Rev. 55, 463–508.

Green, A.R., Gabrielsson, J., Marsden, C.A., Fone, K.C., 2009. MDMA: on the translation from rodent to human dosing. Psychopharmacology 204, 375–378. https://doi.org/10.1007/s00213-008-1453-8.

Hall, A.P., Henry, J.A., 2006 Jun. Acute toxic effects of 'Ecstasy' (MDMA) and related compounds: overview of pathophysiology and clinical management. Br J Anaesth 96 (6), 678–685. https://doi.org/10.1093/bja/ael078.

Halliwell, B., 1992. Reactive oxygen species and the central nervous system. J. Neurochem. 59, 1609–1623. https://doi.org/10.1111/j.1471-4159.1992.tb10990.x. Erratum in: J Neurochem. 2012 Mar;120(5):850.

Halpin, L.E., Collins, S.A., Yamamoto, B.K., 2014. Neurotoxicity of methamphetamine and 3,4-methylenedioxymethamphetamine. Life Sci. 97, 37–44. https://doi.org/10.1016/j.lfs.2013.07.014.

Harding, J.D., 2017. Nonhuman primates and translational research: progress, opportunities, and challenges. ILAR J. 58, 141–150. https://doi.org/10.1093/ilar/ilx033.

Herndon, J.M., Cholanians, A.B., Lau, S.S., Monks, T.J., 2014a. Glial cell response to 3,4-(+/−)-methylenedioxymethamphetamine and its metabolites. Toxicol. Sci. 138, 130–138. https://doi.org/10.1093/toxsci/kft275.

Herndon, J.M., Cholanians, A.B., Lizarraga, L.E., Lau, S.S., Monks, T.J., 2014b. Catechol-o-methyltransferase and 3,4-({+/−})-methylenedioxymethamphetamine toxicity. Toxicol. Sci. 139, 162–173. https://doi.org/10.1093/toxsci/kfu035.

Heuther, G., Reimer, A., Schmidt, F., Schuff-Werner, P., Brudny, M.M., 1990. Oxidation of the indole nucleus of 5-hydroxytryptamine and formation of dimers in the presence of peroxidase and H2O2. J. Neural Transm. Suppl. 32, 249–257. https://doi.org/10.1007/978-3-7091-9113-2_35.

Holze, F., Vizeli, P., Müller, F., Ley, L., Duerig, R., Varghese, N., Eckert, A., Borgwardt, S., Liechti, M.E., 2020. Distinct acute effects of LSD, MDMA, and D-amphetamine in healthy subjects. Neuropsychopharmacology. 45, 462–471. https://doi.org/10.1038/s41386-019-0569-3.

Insel, T.R., Battaglia, G., Johannessen, J.N., Marra, S., De Souza, E.B., 1989. 3,4-Methylenedioxymethamphetamine ("ecstasy") selectively destroys brain serotonin terminals in rhesus monkeys. J. Pharmacol. Exp. Ther. 249, 713–720.

Iravani, M.M., Asari, D., Patel, J., Wieczorek, W.J., Kruk, Z.L., 2000. Direct effects of 3,4-methylenedioxymethamphetamine (MDMA) on serotonin or dopamine release and uptake in the caudate putamen, nucleus accumbens, substantia nigra pars reticulata, and the dorsal raphé nucleus slices. Synapse. 36, 275–285. https://doi.org/10.1002/(SICI)1098-2396(20000615)36:4<275::AID-SYN4>3.0.CO;2-#.

Itzhak, Y., Ali, S.F., 2006. Role of nitrergic system in behavioral and neurotoxic effects of amphetamine analogs. Pharmacol. Ther. 109, 246–262.

Jahns, F.P., Pineau Mitchell, A., Auzinger, G., 2018. Too hot to handle: a case report of extreme pyrexia after MDMA ingestion. Ther. Hypothermia Temp. Manag. 8, 173–175.

Jayanthi, S., Ladenheim, B., Andrews, A.M., Cadet, J.L., 1999. Overexpression of human copper/zinc superoxide dismutase in transgenic mice attenuates oxidative stress caused by methylenedioxymethamphetamine (ecstasy). Neuroscience. 91, 1379–1387. https://doi.org/10.1016/S0306-4522(98)00698-8.

Kettenmann, H., Kirchhoff, F., Verkhratsky, A., 2013. Microglia: new roles for the synaptic stripper. Neuron. 77, 10–18. https://doi.org/10.1016/j.neuron.2012.12.023.

Khairnar, A., Plumitallo, A., Frau, L., Schintu, N., Morelli, M., 2010. Caffeine enhances astroglia and microglia reactivity induced by 3,4-methylenedioxymethamphetamine ('ecstasy') in mouse brain. Neurotox. Res. 17, 435–439. https://doi.org/10.1007/s12640-009-9125-y.

Kish, S.J., 2002 Apr. How strong is the evidence that brain serotonin neurons are damaged in human users of ecstasy? Pharmacol Biochem Behav 71 (4), 845–855. https://doi.org/10.1016/s0091-3057(01)00708-0.

Kish, S.J., 2003. What is the evidence that ecstasy (MDMA) can cause Parkinson's disease? Mov. Disord. 18, 1219–1223. https://doi.org/10.1002/mds.10643.

Kivell, B., Day, D., Bosch, P., Schenk, S., Miller, J., 2010. MDMA causes a redistribution of serotonin transporter from the cell surface to the intracellular compartment by a mechanism independent of phospho-p38-mitogen activated protein kinase activation. Neuroscience. 168, 82–95. https://doi.org/10.1016/j.neuroscience.2010.03.018.

Kreutzberg, G.W., 1996. Microglia: a sensor for pathological events in the CNS. Trends Neurosci. 19, 312–318. https://doi.org/10.1016/0166-2236(96)10049-7.

Kuhn, D.M., Francescutti-Verbeem, D.M., Thomas, D.M., 2006. Dopamine quinones activate microglia and induce a neurotoxic gene expression profile: relationship to methamphetamine-induced nerve ending damage. Ann. N. Y. Acad. Sci. 1074, 31–41. https://doi.org/10.1196/annals.1369.003.

Kuniyoshi, S.M., Jankovic, J., 2003. MDMA and parkinsonism. N. Engl. J. Med. 349, 96–97. https://doi.org/10.1056/NEJMc030208.

LaVoie, M.J., Hastings, T.G., 1999. Dopamine quinone formation and protein modification associated with the striatal neurotoxicity of methamphetamine: evidence against a role for extracellular dopamine. J. Neurosci. 19, 1484–1491.
Liddelow, S.A., Barres, B.A., 2017. Reactive astrocytes: production, function, and therapeutic potential. Immunity. 46, 957–967. https://doi.org/10.1016/j.immuni.2017.06.006.
Liddelow, S., Guttenplan, K., Clarke, L., et al., 2017. Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541, 481–487. https://doi.org/10.1038/nature21029.
Liechti, M.E., Baumann, C., Gamma, A., Vollenweider, F.X., 2000. Acute psychological effects of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") are attenuated by the serotonin uptake inhibitor citalopram. Neuropsychopharmacology. 22, 513–521. https://doi.org/10.1016/S0893-133X(99)00148-7.
Logan, B.J., Laverty, R., Sanderson, W.D., Yee, Y.B., 1988. Differences between rats and mice in MDMA (methylenedioxymethylamphetamine) neurotoxicity. Eur. J. Pharmacol. 152, 227–234. https://doi.org/10.1016/0014-2999(88)90717-0.
Lopez-Rodriguez, A.B., Llorente-Berzal, A., Garcia-Segura, L.M., Viveros, M.P., 2014. Sex-dependent long-term effects of adolescent exposure to THC and/or MDMA on neuroinflammation and serotoninergic and cannabinoid systems in rats. Br. J. Pharmacol. 171, 1435–1447. https://doi.org/10.1111/bph.12519, 38:81-87.
Lyles, J., Cadet, J.L., 2003. Methylenedioxymethamphetamine (MDMA, ecstasy) neurotoxicity: cellular and molecular mechanisms. Brain Res. Rev. 42, 155–168.
Malberg, J.E., Sabol, K.E., Seiden, L.S., 1996. Co-administration of MDMA with drugs that protect against MDMA neurotoxicity produces different effects on body temperature in the rat. J. Pharmacol. Exp. Ther. 278, 258–267.
McCann, U.D., Ridenour, A., Shaham, Y., Ricaurte, G.A., 1994. Serotonin neurotoxicity after (+/−)3,4-methylenedioxymethamphetamine (MDMA; "ecstasy"): a controlled study in humans. Neuropsychopharmacology. 10, 129–138. https://doi.org/10.1038/npp.1994.15.
Mead, J., Parrott, A., 2020. Mephedrone and MDMA: a comparative review. Brain Res. 1735, 146740. https://doi.org/10.1016/j.brainres.2020.146740.
Mehdizadeh, M., Dabaghian, F., Nejhadi, A., Fallah-Huseini, H., Choopani, S., Shekarriz, N., Molavi, N., Basirat, A., Mohammadzadeh Kazorgah, F., Samzadeh-Kermani, A., Soleimani Asl, S., 2012. *Zingiber officinale* alters 3,4-methylenedioxy-methamphetamine-induced neurotoxicity in rat brain. Cell J. 14, 177–184.
Meiser, J., Weindl, D., Hiller, K., 2013. Complexity of dopamine metabolism. Cell Commun. Signal. 11, 34. https://doi.org/10.1186/1478-811X-11-34.
Miller, R.T., Lau, S.S., Monks, T.J., 1996. Effects of intracerebroventricular administration of 5-(glutathion-S-yl)-alpha-methyldopamine on brain dopamine, serotonin, and norepinephrine concentrations in male Sprague-Dawley rats. Chem. Res. Toxicol. 9, 457–465. https://doi.org/10.1021/tx9501546.
Miller, R.T., Lau, S.S., Monks, T.J., 1997. 2,5-Bis-(glutathion-S-yl)-alpha-methyldopamine, a putative metabolite of (+/−)-3,4-methylenedioxyamphetamine, decreases brain serotonin concentrations. Eur. J. Pharmacol. 323, 173–180. https://doi.org/10.1016/s0014-2999(97)00044-7.
Millot, M., Saga, Y., Duperrier, S., Météreau, E., Beaudoin-Gobert, M., Sgambato, V., 2020. Prior MDMA administration aggravates MPTP-induced parkinsonism in macaque monkeys. Neurobiol. Dis. 134, 104643. https://doi.org/10.1016/j.nbd.2019.104643.
Mintzer, S., Hickenbottom, S., Gilman, S., 1999. Parkinsonism after taking ecstasy. N. Engl. J. Med. 340, 1443. https://doi.org/10.1056/nejm199905063401817.
Molliver, M.E., Berger, U.V., Mamounas, L.A., Molliver, D.C., O'Hearn, E., Wilson, M.A., 1990. Neurotoxicity of MDMA and related compounds: anatomic studies. Ann. N. Y. Acad. Sci. 600, 649–661 discussion 661-4. https://doi.org/10.1111/j.1749-6632.1990.tb16916.x.
Moncada, S., Bolaños, J.P., 2006. Nitric oxide, cell bioenergetics and neurodegeneration. J. Neurochem. 97, 1676–1689.
Moratalla, R., Khairnar, A., Simola, N., Granado, N., García-Montes, J.R., Porceddu, P.F., Tizabi, Y., Costa, G., Morelli, M., 2017. Amphetamine-related drugs neurotoxicity in humans and in experimental animals: main mechanisms. Prog. Neurobiol. 155, 149–170.
Mueller, M., Yuan, J., Maldonado Adrian, C., McCann, U.D., Ricaurte, G.A., 2011. Inhibition of 3,4-methylenedioxymethamphetamine metabolism leads to marked decrease in 3,4-dihydroxymethamphetamine formation but no change in serotonin neurotoxicity: implications for mechanisms of neurotoxicity. Synapse. 65, 983–990. https://doi.org/10.1002/syn.20925.
Mueller, M., Maldonado-Adrian, C., Yuan, J., McCann, U.D., Ricaurte, G.A., 2013. Studies of (±)-3,4-methylenedioxymethamphetamine (MDMA) metabolism and disposition in rats and mice: relationship to neuroprotection and neurotoxicity profile. J. Pharmacol. Exp. Ther. 344, 479–488. https://doi.org/10.1124/jpet.112.201699.
Müeller, F., Lenz, C., Steiner, M., Dolder, P.C., Walter, M., Lang, U.E., Liechti, M.E., Borgwardt, S., 2016. Neuroimaging in moderate MDMA use: a systematic review. Neurosci. Biobehav. Rev. 62, 21–34.
Müller, F., Brändle, R., Liechti, M.E., Borgwardt, S., 2019. Neuroimaging of chronic MDMA ("ecstasy") effects: a meta-analysis. Neurosci. Biobehav. Rev. 96, 10–20.
Nash, J.F., 1990. Ketanserin pretreatment attenuates MDMA-induced dopamine release in the striatum as measured by in vivo microdialysis. Life Sci. 47, 2401–2408. https://doi.org/10.1016/0024-3205(90)90484-9.
Orio, L., O'Shea, E., Sanchez, V., Pradillo, J.M., Escobedo, I., Camarero, J., Moro, M.A., Green, A.R., Colado, M.I., 2004. 3,4-Methylenedioxymethamphetamine increases interleukin-1beta levels and activates microglia in rat brain: studies on the relationship with acute hyperthermia and 5-HT depletion. J. Neurochem. 89, 1445–1453. https://doi.org/10.1111/j.1471-4159.2004.02443.x.
O'Shea, E., Esteban, B., Camarero, J., Green, A.R., Colado, M.I., 2001. Effect of GBR 12909 and fluoxetine on the acute and long term changes induced by MDMA ('ecstasy') on the 5-HT and dopamine concentrations in mouse brain. Neuropharmacology 40, 65–74.
O'Suilleabhain, P., Giller, C., 2003. Rapidly progressive parkinsonism in a self-reported user of ecstasy and other drugs. Mov. Disord. 18, 1378–1381. https://doi.org/10.1002/mds.10623.
Pacifici, R., Zuccaro, P., Farre, M., Pichini, S., Di Carlo, S., Roset, P.N., Ortuño, J., Segura, J., de la Torre, R., 1999. Immunomodulating properties of MDMA alone and in combination with alcohol: a pilot study. Life Sci. 65, PL309–PL316.
Pacifici, R., Zuccaro, P., Farre, M., Pichini, S., Di Carlo, S., Roset, P.N., Ortuño, J., Pujadas, M., Bacosi, A., Menoyo, E., Segura, J., de la Torre, R., 2001a. Effects of repeated doses of MDMA ('ecstasy') on cell-mediated immune response in humans. Life Sci. 69, 2931–2941.
Pacifici, R., Zuccaro, P., Hernandez Lopez, C., Pichini, S., Di Carlo, S., Farre, M., Roset, P. N., Ortuño, J., Segura, J., de la Torre, R., 2001b. Acute effects of 3,4-methylene-dioxymethamphetamine alone and in combination with ethanol on the immune system in humans. J. Pharmacol. Exp. Ther. 296, 207–215.
Pacifici, R., Zuccaro, P., Farre, M., Pichini, S., Di Carlo, S., Roset, P.N., Palmi, I., Ortuño, J., Menoyo, E., Segura, J., de la Torre, R., 2002. Cell-mediated immune response in MDMA users after repeated dose administration: studies in controlled versus noncontrolled settings. Ann. N. Y. Acad. Sci. 965, 421–433.
Pantoni, M.M., Anagnostaras, S.G., 2019. Cognitive effects of MDMA in laboratory animals: a systematic review focusing on dose. Pharmacol. Rev. 71, 413–449. https://doi.org/10.1124/pr.118.017087. Erratum in: Pharmacol. Rev. 2021 Apr;73 (2):729.
Pardo-Lozano, R., Farré, M., Yubero-Lahoz, S., O'Mathúna, B., Torrens, M., Mustata, C., Pérez-Mañá, C., Langohr, K., Cuyàs, E., Carbó, M.l., de la Torre, R., 2012. Clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"): the influence of gender and genetics (CYP2D6, COMT, 5-HTT). PLoS One 7, e47599. https://doi.org/10.1371/journal.pone.0047599.
Parrott, A.C., 2012. MDMA and temperature: a review of the thermal effects of 'Ecstasy' in humans. Drug Alcohol Depend. 121, 1–9. https://doi.org/10.1016/j.drugalcdep.2011.08.012.
Peraile, I., Torres, E., Mayado, A., Izco, M., Lopez-Jimenez, A., Lopez-Moreno, J.A., Colado, M.I., O'Shea, E., 2010. Dopamine transporter down-regulation following repeated cocaine: implications for 3,4-methylenedioxymethamphetamine-induced acute effects and long-term neurotoxicity in mice. Br. J. Pharmacol. 159, 201–211. https://doi.org/10.1111/j.1476-5381.2009.00522.x.
Peraile, I., Granado, N., Torres, E., Gutiérrez-López, M.D., Moratalla, R., Colado, M.I., O'Shea, E., 2013. Cocaine potentiates MDMA-induced oxidative stress but not dopaminergic neurotoxicity in mice: implications for the pathogenesis of free radical-induced neurodegenerative disorders. Psychopharmacology 230, 125–135. https://doi.org/10.1007/s00213-013-3142-5.
Piper, B.J., Meyer, J.S., 2004. Memory deficit and reduced anxiety in young adult rats given repeated intermittent MDMA treatment during the periadolescent period. Pharmacol. Biochem. Behav. 79, 723–731. https://doi.org/10.1016/j.pbb.2004.10.001.
Price, J.S., Shear, P., Lisdahl, K.M., 2014. Ecstasy exposure & gender: examining components of verbal memory functioning. PLoS One 9, e115645. https://doi.org/10.1371/journal.pone.0115645.
Puerta, E., Hervias, I., Goñi-Allo, B., Zhang, S.F., Jordán, J., Starkov, A.A., Aguirre, N., 2010. Methylenedioxymethamphetamine inhibits mitochondrial complex I activity in mice: a possible mechanism underlying neurotoxicity. Br. J. Pharmacol. 160, 233–245. https://doi.org/10.1111/j.1476-5381.2010.00663.x.
Quinton, M.S., Yamamoto, B.K., 2006. Causes and consequences of methamphetamine and MDMA toxicity. AAPS J. 8, E337–E347. https://doi.org/10.1007/BF02854904.
Redza-Dutordoir, M., Averill-Bates, D.A., 2016. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim. Biophys. Acta 1863, 2977–2992. https://doi.org/10.1016/j.bbamcr.2016.09.012.
Reveron, M.E., Monks, T.J., Duvauchelle, C.L., 2005. Age-dependent (+)MDMA-mediated neurotoxicity in mice. Neurotoxicology 26, 1031–1040.
Ricaurte, G.A., McCann, U.D., 1992. Neurotoxic amphetamine analogues: effects in monkeys and implications for humans. Ann. N. Y. Acad. Sci. 648, 371–382.
Ricaurte, G.A., Forno, L.S., Wilson, M.A., DeLanney, L.E., Irwin, I., Molliver, M.E., Langston, J.W., 1988. (+/−)3,4-Methylenedioxymethamphetamine selectively damages central serotonergic neurons in nonhuman primates. JAMA 260, 51–55.
Rossi, D., 2015. Astrocyte physiopathology: at the crossroads of intercellular networking, inflammation and cell death. Prog. Neurobiol. 130, 86–120. https://doi.org/10.1016/j.pneurobio.2015.04.003.
Ruiz-Medina, J., Ledent, C., Carretón, O., Valverde, O., 2011. The A2a adenosine receptor modulates the reinforcement efficacy and neurotoxicity of MDMA. J. Psychopharmacol. 25, 550–564. https://doi.org/10.1177/0269881110389210.
Scheffel, U., Szabo, Z., Mathews, W.B., Finley, P.A., Dannals, R.F., Ravert, H.T., Szabo, K., Yuan, J., Ricaurte, G.A., 1998. In vivo detection of short- and long-term MDMA neurotoxicity–a positron emission tomography study in the living baboon brain. Synapse 29, 183–192.
Schilt, T., Koeter, M.W., Smal, J.P., Gouwetor, M.N., van den Brink, W., Schmand, B., 2010. Long-term neuropsychological effects of ecstasy in middle-aged ecstasy/polydrug users. Psychopharmacology 207, 583–591. https://doi.org/10.1007/s00213-009-1688-z.
Schmidt, C.J., 1989. Acute and long-term neurochemical effects of methylenedioxymethamphetamine in the rat. NIDA Res. Monogr. 94, 179–195.
Schmidt, C.J., Taylor, V.L., 1987. Depression of rat brain tryptophan hydroxylase activity following the acute administration of methylenedioxymethamphetamine. Biochem. Pharmacol. 36, 4095–4102. https://doi.org/10.1016/0006-2952(87)90566-1.

Scuderi, C., Stecca, C., Iacomino, A., Steardo, L., 2013. Role of astrocytes in major neurological disorders: the evidence and implications. IUBMB Life 65, 957–961. https://doi.org/10.1002/iub.1223.

Shankaran, M., Yamamoto, B.K., Gudelsky, G.A., 1999a. Mazindol attenuates the 3,4-methylenedioxymethamphetamine-induced formation of hydroxyl radicals and long-term depletion of serotonin in the striatum. J. Neurochem. 72, 2516–2522.

Shankaran, M., Yamamoto, B.K., Gudelsky, G.A., 1999b. Involvement of the serotonin transporter in the formation of hydroxyl radicals induced by 3,4-methylenedioxymethamphetamine. Eur. J. Pharmacol. 385, 103–110.

Shih, J.H., Ma, K.H., Chen, C.F., Cheng, C.Y., Pao, L.H., Weng, S.J., Huang, Y.S., Shiue, C. Y., Yeh, M.K., Li, I.H., 2016. Evaluation of brain SERT occupancy by resveratrol against MDMA-induced neurobiological and behavioral changes in rats: a 4-[^{18}F]-ADAM/small-animal PET study. Eur. Neuropsychopharmacol. 26, 92–104. https://doi.org/10.1016/j.euroneuro.2015.11.001.

Shokry, I.M., Shields, C.J., Callanan, J.J., Ma, Z., Tao, R., 2019. Differential role of dose and environment in initiating and intensifying neurotoxicity caused by MDMA in rats. BMC Pharmacol. Toxicol. 20, 47. https://doi.org/10.1186/s40360-019-0326-6.

Siegel, R.K., 1986. MDMA. Nonmedical use and intoxication. J. Psychoactive Drugs 18, 349–354. https://doi.org/10.1080/02791072.1986.10472368.

Smith, Y., Galvan, A., 2018. Non-human primate research of basal ganglia and movement disorders: advances and challenges. J. Neural Transm. 125 (3), 275–278. https://doi.org/10.1007/s00702-018-1849-5.

Sofroniew, M.V., Vinters, H.V., 2010. Astrocytes: biology and pathology. Acta Neuropathol. 119, 7–35. https://doi.org/10.1007/s00401-009-0619-8.

Sprague, J.E., Nichols, D.E., 1995. The monoamine oxidase-B inhibitor L-deprenyl protects against 3,4-methylenedioxymethamphetamine-induced lipid peroxidation and long-term serotonergic deficits. J. Pharmacol. Exp. Ther. 273, 667–673.

Sprague, J.E., Everman, S.L., Nichols, D.E., 1998. An integrated hypothesis for the serotonergic axonal loss induced by 3,4-methylenedioxymethamphetamine. Neurotoxicology. 19, 427–441.

Stefanatos, R., Sanz, A., 2018. The role of mitochondrial ROS in the aging brain. FEBS Lett. 592, 743–758. https://doi.org/10.1002/1873-3468.12902.

Stokes, A.H., Hastings, T.G., Vrana, K.E., 1999. Cytotoxic and genotoxic potential of dopamine. J. Neurosci. Res. 55, 659–665. https://doi.org/10.1002/(SICI)1097-4547(19990315)55:6<659::AID-JNR1>3.0.CO;2-C.

Stone, D.M., Hanson, G.R., Gibb, J.W., 1987. Differences in the central serotonergic effects of methylenedioxymethamphetamine (MDMA) in mice and rats. Neuropharmacology. 26, 1657–1661. https://doi.org/10.1016/0028-3908(87)90017-7.

Taghizadeh, G., Pourahmad, J., Mehdizadeh, H., Foroumadi, A., Torkaman-Boutorabi, A., Hassani, S., Naserzadeh, P., Shariatmadari, R., Gholami, M., Rouini, M.R., Sharifzadeh, M., 2016. Protective effects of physical exercise on MDMA-induced cognitive and mitochondrial impairment. Free Radic. Biol. Med. 99, 11–19. https://doi.org/10.1016/j.freeradbiomed.2016.07.018.

Tai, Y.F., Hoshi, R., Brignell, C.M., Cohen, L., Brooks, D.J., Curran, H.V., Piccini, P., 2011. Persistent nigrostriatal dopaminergic abnormalities in ex-users of MDMA ('Ecstasy'): an 18F-dopa PET study. Neuropsychopharmacology 36, 735–743.

Takamatsu, Y., Shiotsuki, H., Kasai, S., Sato, S., Iwamura, T., Hattori, N., Ikeda, K., 2011. Enhanced hyperthermia induced by MDMA in Parkin knockout mice. Curr. Neuropharmacol. 9, 96–99. https://doi.org/10.2174/157015911795016985.

Taraska, T., Finnegan, K.T., 1997. Nitric oxide and the neurotoxic effects of methamphetamine and 3,4-methylenedioxymethamphetamine. J. Pharmacol. Exp. Ther. 280, 941–947.

Thomas, D.M., Dowgiert, J., Geddes, T.J., Francescutti-Verbeem, D., Liu, X., Kuhn, D.M., 2004. Microglial activation is a pharmacologically specific marker for the neurotoxic amphetamines. Neurosci. Lett. 367, 349–354. https://doi.org/10.1016/j.neulet.2004.06.065.

Todd, G., Noyes, C., Flavel, S.C., Della Vedova, C.B., Spyropoulos, P., Chatterton, B., Berg, D., White, J.M., 2013. Illicit stimulant use is associated with abnormal substantia nigra morphology in humans. PLoS One 8, e56438. https://doi.org/10.1371/journal.pone.0056438.

Touriño, C., Zimmer, A., Valverde, O., 2010. THC prevents MDMA neurotoxicity in mice. PLoS One 5, e9143. https://doi.org/10.1371/journal.pone.0009143.

Turrens, J.F., 2003. Mitochondrial formation of reactive oxygen species. J. Physiol. 552, 335–344. https://doi.org/10.1113/jphysiol.2003.049478.

Vegting, Y., Reneman, L., Booij, J., 2016. The effects of ecstasy on neurotransmitter systems: a review on the findings of molecular imaging studies. Psychopharmacology 233, 3473–3501.

Vercoulen, E., Hondebrink, L., 2021. Combining ecstasy and ethanol: higher risk for toxicity? A review. Crit. Rev. Toxicol. 51, 1–14. https://doi.org/10.1080/10408444.2020.1867822.

Verrico, C.D., Miller, G.M., Madras, B.K., 2007. MDMA (ecstasy) and human dopamine, norepinephrine, and serotonin transporters: implications for MDMA-induced neurotoxicity and treatment. Psychopharmacology 189, 489–503. https://doi.org/10.1007/s00213-005-0174-5.

Vizeli, P., Liechti, M.E., 2019. No influence of dopamine system gene variations on acute effects of MDMA. Front. Psychiatry. 10, 755. https://doi.org/10.3389/fpsyt.2019.00755.

Wang, X., Baumann, M.H., Xu, H., Morales, M., Rothman, R.B., 2005. (+/−)-3,4-Methylenedioxymethamphetamine administration to rats does not decrease levels of the serotonin transporter protein or alter its distribution between endosomes and the plasma membrane. J. Pharmacol. Exp. Ther. 314, 1002–1012. https://doi.org/10.1124/jpet.105.088476.

Whitehead, R.E., Ferrer, J.V., Javitch, J.A., Justice, J.B., 2001. Reaction of oxidized dopamine with endogenous cysteine residues in the human dopamine transporter. J. Neurochem. 76, 1242–1251.

Yang, L., Mih, N., Anand, A., Park, J.H., Tan, J., Yurkovich, J.T., Monk, J.M., Lloyd, C.J., Sandberg, T.E., Seo, S.W., Kim, D., Sastry, A.V., Phaneuf, P., Gao, Y., Broddrick, J.T., Chen, K., Heckmann, D., Szubin, R., Hefner, Y., Feist, A.M., Palsson, B.O., 2019. Cellular responses to reactive oxygen species are predicted from molecular mechanisms. Proc. Natl. Acad. Sci. U. S. A. 116, 14368–14373. https://doi.org/10.1073/pnas.1905039116.

Zafar, K.S., Siegel, D., Ross, D., 2006. A potential role for cyclized quinones derived from dopamine, DOPA, and 3,4-dihydroxyphenylacetic acid in proteasomal inhibition. Mol. Pharmacol. 70, 1079–1086. https://doi.org/10.1124/mol.106.024703.

Zhou, Z.D., Lim, T.M., 2009. Dopamine (DA) induced irreversible proteasome inhibition via DA derived quinones. Free Radic. Res. 43, 417–430. https://doi.org/10.1080/10715760902801533.

Zhou, J.F., Chen, P., Zhou, Y.H., Zhang, L., Chen, H.H., 2003. 3,4-Methylenedioxymethamphetamine (MDMA) abuse may cause oxidative stress and potential free radical damage. Free Radic. Res. 37, 491–497. https://doi.org/10.1080/1071576031000076286.

Zou, J.Y., Crews, F.T., 2005. TNF alpha potentiates glutamate neurotoxicity by inhibiting glutamate uptake in organotypic brain slice cultures: neuroprotection by NF kappa B inhibition. Brain Res. 1034, 11–24. https://doi.org/10.1016/j.brainres.2004.11.014.