



## A review on the mitochondrial toxicity of “ecstasy” (3,4-methylenedioxymethamphetamine, MDMA)



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### ABSTRACT

3,4-Methylenedioxymethamphetamine (MDMA or “ecstasy”) is a drug of abuse used by millions worldwide. MDMA human abuse and dependence is well described, but addictive properties are not always consistent among studies. This amphetamine is a substrate type releaser, binding to monoamine transporters, leading to a pronounced release of serotonin and noradrenaline and to a minor extent dopamine. The toxicity of MDMA is well studied at the pre-clinical level, with neurotoxicity and hepatotoxicity being particularly described.

In this review, we describe the most relevant MDMA effects at the mitochondrial level found in *in vitro* and *in vivo* models, these later conducted in mice and rats. Most of these reports focus on the mitochondria of brain or liver. In *in vitro* models, MDMA causes depletion of ATP levels and inhibition of mitochondrial complex I and III, loss in mitochondrial membrane potential ( $\Delta\Psi_m$ ) and induction of mitochondrial permeability transition. The involvement of mitochondria in the apoptotic cell death evoked by MDMA has also been shown, such as the release of cytochrome *c*. Additionally, MDMA or its metabolites impaired mitochondrial trafficking and increased the fragmentation of axonal mitochondria. In animal studies, MDMA decreased mitochondrial complex I activity and decreased ATP levels. Moreover, MDMA-evoked oxidative stress has been shown to cause deletion on mitochondrial DNA and impairment in mitochondrial protein synthesis.

Although the concentrations and doses used in some studies do not always correlate to the human scenario, the mitochondrial abnormalities evoked by MDMA are well described and are in part responsible for its mechanism of toxicity.

## 1. Introduction

### 1.1. History and epidemiology

3,4-Methylenedioxymethamphetamine (MDMA or “ecstasy”) is a psychotropic drug regularly used by millions worldwide. Chemically it is a ring-substituted amphetamine derivative structurally related to the monoamine neurotransmitters (Fig. 1). Records indicate that it was first synthesized in 1912 by the German pharmaceutical company Merck without any intention of using it therapeutically, but as a

precursor for other drugs (Freudenmann et al., 2006). It was later rediscovered in the 1970s by United States psychotherapists who intended to use MDMA therapeutically, in combination with psychotherapy (Freudenmann et al., 2006; Passie and Benzenhöfer, 2016). Case reports from that period described its therapeutic benefits, although no clinical trials were conducted at that time, but more recently, from 2004 to 2017, six phase 2 randomized trials of MDMA-assisted psychotherapy for treatment of post-traumatic stress disorder (PTSD) were conducted (Mithoefer et al., 2019), revealing therapeutic benefits. Following these promising results a new phase

**Abbreviations:** 5-HT, Serotonin; MAO, Monoamine oxidase; MDMA, 3,4-Methylenedioxymethamphetamine; MPT, Mitochondrial permeability transition; NA, Noradrenaline; PST, Post-traumatic stress disorder; ROS, Reactive oxygen species; SERT, Serotonin transporter; UCP-3, Uncoupling protein-3;  $\Delta\Psi_m$ , Mitochondrial membrane potential.

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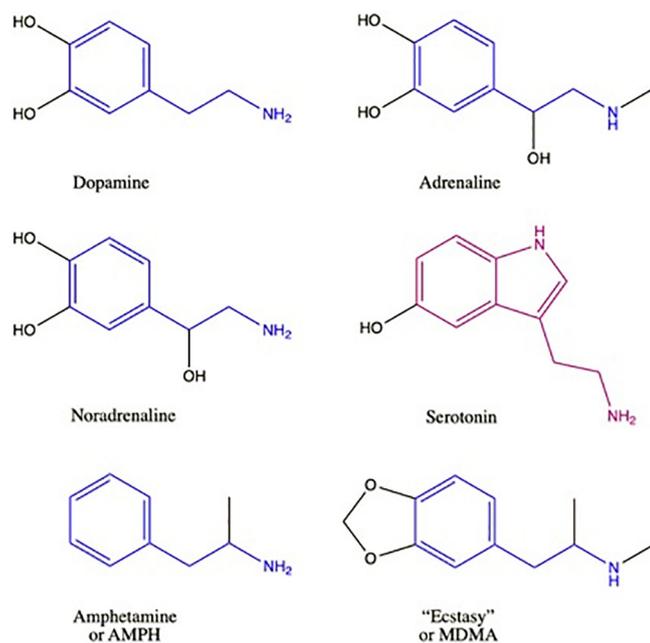
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**Fig. 1.** Chemical structures of monoamine brain neurotransmitters and of amphetamine and “ecstasy”. The 2-phenylethylamine moiety is shown in blue and the tryptamine backbone in serotonin is highlighted in purple. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3 trial showed that MDMA-assisted therapy is highly efficacious in individuals with severe PTSD (Mitchell et al., 2021). It remains, however, to be proven if MDMA will have any role in therapeutic settings, as not only its efficacy but also the therapeutic safety of the drug remains to be established.

In San Francisco, in the 1970s drug dealers coined the name of “ecstasy” for the drug, for marketing purposes (Passie and Benzenhöfer, 2016). The use of MDMA spread in the 1980s to Europe and thereafter to the rest of the world (Freudenmann et al., 2006). MDMA abuse is mainly associated with recreational nightlife settings, and is consumed in the form of tablets (rarely capsules or powder). The United Nations estimate that nearly 20 million people have used MDMA in 2020, corresponding to 0.4 per cent of the global population aged 15–64 (UNODC, 2021). In accordance to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), in 2019 the average drug content of MDMA tablets seized in Europe ranged from 118 to 210 mg per tablet (EMCDDA, 2021). Surveys in Europe show that 2 million young adults (aged 15–34) used MDMA in the last year, but prevalence estimates for those aged 15–24 years are higher with 2.2 % (1.0 million) to have used MDMA in the last year (EMCDDA, 2021).

## 1.2. Pharmacology

MDMA acts both in the central and peripheral nervous system, targeting monoaminergic neurons. In particular, MDMA is a substrate type releaser, targeting particularly the transporters of noradrenaline (NA) and serotonin (5-HT), promoting its neuronal release (Hysek et al., 2012; Luethi et al., 2019). MDMA binds to the plasma membrane monoamine transporters, and is transported into the cell and thereafter stimulates the neurotransmitter release via the same transporters. Like other amphetamine related compounds, MDMA is more potent at stimulating NA release, but it differs from amphetamine or methamphetamine. The last preferentially target dopamine (DAT) transporters, while MDMA displays higher affinity to 5-HT transporter (SERT), thereby stimulating a non-vesicular release of 5-HT and other

monoamines to the synaptic clefts (Baumann et al., 2014; Zsilla et al., 2018; Luethi et al., 2019). MDMA also displays a strong affinity, acting as an agonist, for monoamine receptors including 5-HT<sub>2A</sub>, 5-HT<sub>1A</sub> or 5-HT<sub>2C</sub>,  $\alpha_{1A}$  and  $\alpha_{2A}$  and also, trace amine-associated receptor (TAAR) receptor 1 (Simmler et al., 2014; Pitts et al., 2017; Luethi et al., 2019). The human effects after MDMA correlate to the pharmacological action of the drug in the monoamine transporters and receptors. For instance, the energy to dance for hours and euphoria are more related to the release of monoamine transmitters, meanwhile, the mild hallucinogenic actions of the drug are related to the 5-HT<sub>2A</sub> receptor agonism (Erritzoe et al., 2011). Drug abusers describe the mood-enhancing properties of MDMA, conveyed in the “3 Es” expression: energy, empathy, and euphoria (Hall and Henry, 2006). MDMA human abuse and dependence is well described, but addictive properties are not always reported in a consistent manner across studies (Cottler et al., 2009). Human and animal studies converge into the idea that higher doses are prone to evoke addiction, meanwhile ultra-low doses (<1 mg/kg) seem safer (Pantoni et al., 2022).

It is important to know the pharmacokinetics of MDMA and the production of liver metabolites to understand the toxic effects of the drug. Pharmacokinetic studies in humans show that MDMA is readily absorbed from the gastrointestinal tract. The onset of action is within 30 min and peak serum levels occur after one to three hours (de la Torre et al., 2000). The drug exhibits non-linear pharmacokinetics in humans and small increases in the dose of MDMA are translated to disproportionate rises in MDMA plasma concentrations. For a dose of 100 mg the elimination half-life is approximately 7 – 8 h (de la Torre et al., 2000).

As stated, MDMA is mainly metabolized in the liver, with approximately 80 % of the drug being transformed in the liver, while 20 % of the dose is excreted unaltered in urine. Hepatic MDMA metabolism generates several metabolites that are known to be more toxic to the brain or liver than the parent compound (de la Torre et al., 2004). MDMA liver metabolism catalyzed by hepatic cytochrome P-450 (CYP) isoenzymes generates 3,4-methylenedioxyamphetamine (MDA), and then through oxidation 3,4-dihydroxymethamphetamine (N-methyl- $\alpha$ -methyl-dopamine (N-Me- $\alpha$ -MeDA)) and 3,4-dihydroxyamphetamine ( $\alpha$ -methyl-dopamine ( $\alpha$ -MeDA)), two extremely reactive catechols (de la Torre et al., 2004). Subsequently phase I metabolites are conjugated with glutathione (GSH) or N-acetylcysteine (NAC), or otherwise metabolized by catechol-O-methyltransferase, sulfotransferase, or uridine diphosphate glucuronosyl transferase phase II enzymes (de la Torre et al., 2004). These conjugates are preferentially excreted in urine. Both oxidative metabolites and conjugates with GSH and NAC are known to reach peripheral organs and promote toxicity, including the brain or liver (Capela et al., 2006; Capela et al., 2007).

## 1.3. Toxicity

The immediate effects that follow the drug intake include mild adverse effects, such as: dry mouth, ataxia, stiffness and pain in the back and limbs, headache, nausea, loss of appetite, blurred vision and mydriasis, insomnia, increased muscle tension that leads to jaw clenching, tooth grinding and restless leg movements (Verheyden et al., 2003). There are also acute cardiovascular effects of MDMA that encompass rises in heart rate, blood pressure and cardiac output (Hall and Henry, 2006). MDMA is viewed as a cardiotoxicant, like other amphetamines, and contraction band necrosis can be observed after MDMA intake in post-mortem analysis (Carvalho et al., 2012; Costa et al., 2013).

Hyperthermia is an event that can potentiate all other toxic events, and is seen as life-threatening (Hall and Henry, 2006; Campbell and Rosner, 2008). Fulminant hyperthermia, with core temperatures higher than 42 °C, which usually precede disseminated intravascular coagulation, rhabdomyolysis, hepatotoxicity, acute renal failure, and (multiple) organ failure that may lead to death (Hall and Henry,

2006; Campbell and Rosner, 2008). The MDMA-related death rate in the 15 to 24-year-old MDMA users in the UK has been estimated to lay from 0.2 to 5.3 per 10,000 (Gore, 1999).

Both hepatotoxicity and nephrotoxicity have been well described, both in animal and human studies. These toxic effects may arise as a direct action of MDMA and its metabolites, or as a consequence of hyperthermia and disseminated intravascular coagulation (Hall and Henry, 2006). Liver dysfunction can be due to extensive and/or focal hepatic necrosis, and might result in fulminant hepatic failure requiring urgent liver transplantation (Hall and Henry, 2006). MDMA has been associated with acute kidney injury most likely secondary to rhabdomyolysis (Campbell and Rosner, 2008). Another potentially fatal complication arising from MDMA intake is hyponatraemia, which seems to arise from stimulation of antidiuretic hormone secretion by the drug and affects mostly females (Campbell and Rosner, 2008; van Dijken et al., 2013).

There are more problematic acute psychiatric and neurological events in MDMA users, that include insomnia, mild hallucinations, anxiety, agitation or even panic attacks, delirium, or brief psychotic episodes (Hall and Henry, 2006). In the next days following drug use, reduced appetite, depression, anxiety, difficulty concentrating, muscle aches and fatigue have been reported (Verheyden et al., 2003). In chronic users depression, anxiety or cognitive impairments have been reported, which are consistent with serotonergic system changes, although whether this reflects neurotoxicity or neuroadaptation remains elusive (Roberts et al., 2018). In addition to these, MDMA also induces the classic symptoms of serotonin syndrome, as a result of the increase in brain serotonin release. A wide number of animal studies, including those with non-human primates, have provided evidences for MDMA neurotoxic actions. MDMA evokes depletion on 5-HT and SERT levels and loss of serotonergic terminals, accompanied by neuronal loss in some particular brain areas in animals (Capela et al., 2009; Song et al., 2010; Steinkellner et al., 2011; Halpin et al., 2014; Costa et al., 2020). In humans, lower levels of SERT binding and metabolites in the cerebrospinal fluid of MDMA users have been found, suggesting serotonergic axonal damage particularly in the thalamus (de Win et al., 2008).

## 2. Search strategy and selection criteria

To address MDMA effects at the mitochondrial level a search on PubMed database was performed on the 9th of July 2021, for articles published from 2000 onwards, with following keywords: (3,4-methylenedioxymethamphetamine OR MDMA OR "ecstasy") in combination with the following search terms (mitochondria OR mitochondrial) and (toxicity OR impairment). We examined related references and selected full-text research articles that were only related to the topic, and particularly searched studies conducted either *in vitro* (15 studies) or *in vivo* with laboratory animals (17 studies) that evaluated MDMA effects to the mitochondria.

## 3. Mitochondrial actions of MDMA

The reports conducted to evaluate the MDMA effects at the mitochondrial level, mostly targeted the brain or liver either *in vitro* or in animals, given the well-known neurotoxic and hepatotoxic effects of MDMA. These reports agree with the toxic actions of MDMA at the mitochondrial level. In fact, abnormal mitochondrial function was also reported with other amphetamines, and was closely associated with deregulation on bioenergetic metabolism associated to the neurotoxic actions of amphetamines, for a review see Barbosa et al. (2015).

Bellow we describe the most relevant findings of these studies dividing them into *in vitro* and *in vivo* reports.

### 3.1. Studies *in vitro*

Most studies that evaluated MDMA mitochondrial actions *in vitro* were conducted either with neuronal or hepatic cells. Investigators piked these cellular models given that they more readily allow to investigate the neurotoxic and hepatotoxic actions of MDMA. A summary of the major findings of these *in vitro* studies is detailed in Table 1.

In general, studies reported decreased ATP levels, following exposure to MDMA or to its metabolites. A decrease in ATP levels might be indicative that the mitochondria, as primary ATP-producing organelles, are targeted by MDMA. In accordance a study reported impaired basal and maximal cellular respiration, with MDMA inhibiting mitochondrial complex I and III (Zhou et al., 2019). Also, studies reported a loss in mitochondrial membrane potential ( $\Delta\Psi_m$ ) and induction of mitochondrial permeability transition (MPT) in mitochondria from different models (Custódio et al., 2010; Dias da Silva et al., 2014; Nakagawa et al., 2017; Popova et al., 2016). The notable exception was a study conducted in neuronally differentiated P19 mouse embryonal carcinoma cells that reported no meaningful differences in the  $\Delta\Psi_m$  (Popova et al., 2016). It is important to highlight as well the important role that MDMA metabolites, in particular the GSH and NAC conjugates, play in MDMA toxic actions, including those at the mitochondrial level. In fact, several *in vitro* studies have proven that MDMA metabolites can be more neurotoxic than the parent compound (Capela et al., 2006; Capela et al., 2007; Ferreira et al., 2013; Barbosa et al., 2014a; Barbosa et al., 2014b).

Of note, studies do not always agree in some findings. One report in freshly isolated rat hepatocytes found that MDMA evoked a decrease in oxygen uptake in state 3 respiration (Nakagawa et al., 2017), meanwhile other study in isolated rat liver mitochondria found that MDMA did not significantly affect state 3 and state 4 respiration rates (Custódio et al., 2010). Besides the difference in the model used in the two studies, there were major differences in the MDMA concentration range. In fact, Nakagawa and co-workers used an MDMA concentration up to 4 mM, while the Custodio and colleagues' work used concentrations up to 2 mM. The concentrations as well as the type of experimental model can greatly influence the results.

Not only energetic changes have been reported. In fact, in neuronal cells deficits in mitochondrial axonal transport of mitochondria were reported following exposure to MDMA. In fact, cultured mice hippocampal neurons exposed to MDMA (1.6 mM) for 45, 90 or 120 min, had a dramatic reduction in the mitochondrial trafficking in a time-dependent manner (Barbosa et al., 2014c). The same authors also reported that MDMA and six of its major metabolites, catechol and conjugated metabolites known to be neurotoxic, each compound at 10  $\mu$ M, impaired mitochondrial trafficking and increased the fragmentation of axonal mitochondria (Barbosa et al., 2014b).

In an attempt to justify MDMA neurotoxic actions, other authors have suggested that the increase in 5-HT release evoked by MDMA in an pro-oxidant environment could lead to oxidation of 5-HT and the formation of the endogenous neurotoxin tryptamine-4,5-dione (Jiang and Dryhurst, 2002). This later compound has shown the ability to inhibit pyruvate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase complexes in rat brain mitochondria (Jiang and Dryhurst, 2002). Moreover, tryptamine-4,5-dione proved to inactivate mitochondrial complex I and complex IV (Jiang and Dryhurst, 2002).

Only two studies evaluated the effects of MDMA in cardiac muscle cells (Tiangco et al., 2005; Zhou et al., 2019). Given the ability of MDMA to cause cardiotoxicity, cardiac muscle cellular models are valuable to study the mechanisms behind this toxicity. These studies generally agree with the findings obtained in hepatic and neuronal models, and depolarization of mitochondrial membranes, depletion on ATP levels and inhibition of mitochondrial complex I and III were reported (Tiangco et al., 2005; Zhou et al., 2019).

**Table 1**Main findings of studies conducted *in vitro* evaluating MDMA effects at the mitochondrial level. Studies are sorted by publication date.

In vitro Model	MDMA and/or metabolites	Concentration range ( $\mu\text{M}$ )	Main findings	Reference
Isolated rat hepatocytes and an hepatic stellate cell line	MDMA	500–5000	Apoptosis correlated with decreased levels of bcl-x(L) and release of cytochrome c from the mitochondria after 8 h of exposure period.	(Montiel-Duarte et al., 2002)
Rat cerebellar granule neuronal cultures	MDMA	1000–5000	Apoptosis correlated with increase in the cytochrome c release from mitochondria after 24 h of exposure.	(Jiménez et al., 2004)
Cultured rat striated cardiac myocytes H9c2	MDMA	1000–10000	MDMA caused depolarization of mitochondrial membranes.	(Tiangco et al., 2005)
Rat cortical neuronal cultures	MDMA, 5-(NAC)-N-Me- $\alpha$ -MeDA, 5-(GSH)-N-Me- $\alpha$ -MeDA	100–400	The metabolite 5-(GSH)-N-Me- $\alpha$ -MeDA, but not MDMA, evoked a decrease in ATP content after 6 h of exposure at normothermic (36.5 °C) and hyperthermic (40 °C) conditions.	(Capela et al., 2007)
Isolated rat liver mitochondria	MDMA	50–2000	MDMA stimulated mitochondrial H <sub>2</sub> O <sub>2</sub> generation in a concentration dependent manner, and protected rat mitochondria against MPT, also did not significantly affect state 3 and state 4 respiration rates, using glutamate/malate or succinate as the respiratory substrates.	(Custódio et al., 2010)
Freshly isolated rat hepatocytes	MDMA	0.312	$\Delta\Psi\text{m}$ was rapidly decreased by MDMA, which was prevented by ROS scavengers and antioxidants. Antioxidants, ROS scavengers, lysosomal inactivators, MPT pore sealing agents, NADPH P450 reductase inhibitor, and inhibitors of reduced CYP2E1 and CYP2D6 prevented all MDMA induced ROS production during a 60 min exposure period.	(Pourahmad et al., 2010)
Rat hippocampal neuronal cultures	MDMA	100–800	In neurons exposed 24 h to MDMA a significant increase in the 67 kDa band of apoptosis inducing factor (AIF) in the mitochondrial fraction, meanwhile there was an increase in cytochrome c in the cytoplasmic fraction.	(Capela et al., 2013)
Immortalized human HepG2 hepatic cells	MDMA	1300	Cells exposed for 24 h to MDMA revealed an elevation of Bax, cleaved Bid, Puma, Bak and Bim protein levels. MDMA reduced ATP levels and disrupted $\Delta\Psi\text{m}$ only in hyperthermic conditions (40.5 °C).	(Dias da Silva et al., 2013)
Immortalized human HepG2 hepatic cells	MDMA	300–1800	Cells exposed 24 h to MDMA revealed a reduction in ATP levels and disrupted $\Delta\Psi\text{m}$ , that were exacerbated by hyperthermic conditions (40.5 °C).	(Dias da Silva et al., 2014)
Mice hippocampal neuronal cultures	MDMA	1600	Neurons exposed 90 min to MDMA dramatically reduced mitochondrial trafficking in a Tau-dependent manner, in which glycogen synthase kinase 3 $\beta$ activity was implicated. Trafficking abnormalities were rescued by over-expression of Mitofusin2 and dynamin-related protein 1, but not of Miro1.	(Barbosa et al., 2014c)
Mice hippocampal neuronal cultures	MDMA, $\alpha$ -MeDA, N-Me- $\alpha$ -MeDA, 5-(GSH)- $\alpha$ -MeDA, 5-(GSH)-N-Me- $\alpha$ -MeDA, 5-(NAC)- $\alpha$ -MeDA, and 5-(NAC)-N-Me- $\alpha$ -MeDA	10	Neurons exposed 24 h to the mixture of MDMA and six of its major metabolites, each compound at 10 $\mu\text{M}$ , impaired mitochondrial trafficking and increased the fragmentation of axonal mitochondria. Furthermore, the overexpression of mitofusin 2 or dynamin-related protein 1 K38A constructs almost completely rescued the trafficking deficits caused by this mixture.	(Barbosa et al., 2014b)
Isolated rat brain mitochondria P <sub>2</sub> fraction	MDMA	1–10	MDMA concentration-dependently inhibited the mitochondrial complex I activity.	(Karuppagounder et al., 2014)
Neuronally differentiated P19 mouse embryonal carcinoma cells	MDMA	1000	MDMA did not alter the $\Delta\Psi\text{m}$ in incubations up to 48 h.	(Popova et al., 2016)
Freshly isolated rat hepatocytes	MDMA	4000	MDMA induced a rapid depletion of intracellular ATP, loss of $\Delta\Psi\text{m}$ and mitochondrial membrane permeability transition disruption, following periods of incubation lower than 2 h. Oxygen uptake in state 3 respiration was decreased, but not state 4.	(Nakagawa et al., 2017)
Mouse skeletal muscle cell line C2C12 myoblasts	MDMA	200–2000	MDMA depleted ATP levels and increased mitochondrial superoxide concentrations in a concentration-dependent manner. Additionally, impaired basal and maximal cellular respiration, inhibiting mitochondrial complex I and III.	(Zhou et al., 2019)

$\Delta\Psi\text{m}$ , mitochondrial membrane potential; MPT, mitochondrial permeability transition;  $\alpha$ -MeDA, alpha-methyl-dopamine; N-Me- $\alpha$ -MeDA, N-methyl-alfa-methyl-dopamine; 5-(GSH)- $\alpha$ -MeDA, 5-(glutathion-S-yl)- $\alpha$ -methyl-dopamine; 5-(NAC)- $\alpha$ -MeDA, 5-(N-acetylcystein-S-yl)- $\alpha$ -methyl-dopamine; 5-(GSH)-N-Me- $\alpha$ -MeDA, 5-(glutathion-S-yl)-N-methyl- $\alpha$ -methyl-dopamine; 5-(NAC)-N-Me- $\alpha$ -MeDA, 5-(N-acetylcystein-S-yl)-N-methyl- $\alpha$ -methyl-dopamine.

Reports also reveal the mitochondrial involvement in MDMA-evoked apoptotic cell death. In cellular models, MDMA evoked an elevation of Bax, cleaved Bid, Puma, Bak and Bim protein levels, which was accompanied by a release of cytochrome c from the mitochondria and cleavage of the apoptosis inducing factor (Montiel-Duarte et al.,

2002; Jiménez et al., 2004; Capela et al., 2013; Dias da Silva et al., 2013). Overall, these reports highlight the mitochondrial involvement in the intrinsic apoptotic pathway after MDMA exposure.

It is worth to mention, that the majority of the *in vitro* experiments reviewed herein use concentrations that are well-above the expected

human brain levels. Additionally, the high micromolar or millimolar concentrations are several fold above the reported affinity of these compounds to monoamine transporters or receptors (that can be found in the nM or low  $\mu$ M range) (Baumann et al., 2014; Simmler et al., 2014; Pitts et al., 2017; Zsilla et al., 2018; Luethi et al., 2019). Nevertheless, these studies are relevant from a mechanistic point of view and can steer future research *in vivo* to more sensitive endpoints, possibly more relevant to the human exposure scenario.

### 3.2. Studies *in vivo*

Reports conducted in mice and rat experimental models in general confirm the *in vitro* findings, ruling for MDMA toxic actions at the mitochondria level. A summary of the major findings of these animal studies is detailed in Table 2.

Generally, animal studies conducted to evaluate MDMA toxicity used doses in a binge schedule, to mimic the repetitive use of the drug in short intervals by abusers. The vast majority of studies were conducted to evaluate MDMA mitochondrial actions in the brain. We could only find three reports studying MDMA mitochondrial actions in the myocardium, skeletal muscle and liver (Gesi et al., 2002; Rusyniak et al., 2005; Moon et al., 2008).

Studies targeting MDMA brain actions, reported a decrease in ATP levels following MDMA exposure, which can be a hallmark of MDMA toxic actions at the mitochondrial level (Taghizadeh et al., 2016; Teixeira-Gomes et al., 2016; Feio-Azevedo et al., 2018).

In agreement, studies have proven that MDMA decreased mitochondrial complex I activity in the brain of mice (Puerta et al., 2010; Barros-Miñones et al., 2015). In rats it has also been shown that MDMA induced the collapse of  $\Delta\Psi_m$  and mitochondrial swelling (Taghizadeh et al., 2016; Taghizadeh et al., 2020a; Taghizadeh et al., 2020b). Given that the main source of ATP in the cell is the mitochondrial electron transport chain, the inhibition of one complex and the collapse of  $\Delta\Psi_m$  can both greatly contribute to the decrease in the generation of ATP.

Mitochondrial electron transport chain is a main location of reactive oxygen species (ROS) production and its dysfunction results in oxidative stress, which plays an important role in many brain pathogenesis. In fact, complexes I and III are main sources of ROS production (Barbosa et al., 2015). Several studies indicate that MDMA administration increases the ROS production and this pro-oxidant effect has been shown to cause deletion on mitochondrial DNA and impairment in mitochondrial protein synthesis (Alves et al., 2007; Alves et al., 2009a; Alves et al., 2009b). Confirming the importance of oxidative stress in the mitochondrial DNA deletions, inhibition of monoamine oxidase B (MAO-B), an enzyme that metabolizes the monoamine neurotransmitters yielding  $H_2O_2$  as a byproduct, could avoid the oxidative damage to DNA (Alves et al., 2007). The contribution of MAO-B to MDMA-induced mitochondrial damage in central nervous system was clearly evidenced, given that the inhibition of this enzyme by selegiline completely protected brain mitochondria against oxidative stress, and prevented mitochondrial DNA deletion and correspondent protein expression (Alves et al., 2007). In addition, acetyl-L-carnitine, which facilitates the transport of long chain free fatty acids across the mitochondrial membrane, enhancing neuronal antioxidant defense, prevented mitochondrial DNA deletions and brain serotonergic neurotoxicity (Alves et al., 2009a). Overall, these results highlight the importance of oxidative stress in the damage inflicted to the mitochondria and in MDMA-induced neurotoxicity.

Two reports evaluated the role of mitochondrial ATP-sensitive potassium channels in the neurotoxicity induced by MDMA. Authors showed that both minoxidil, a potassium ATP channel activator, and sildenafil, a phosphodiesterase 5 inhibitor, protected against MDMA neurotoxic actions to serotonergic neurons via activation of mitochondrial ATP-sensitive potassium channel opening (Goñi-Allo et al., 2008; Puerta et al., 2009). Activation of mitochondrial ATP-sensitive potas-

sium channels seems to be neuroprotective via the attenuation of ROS production (Puerta et al., 2009).

Reports focusing in peripheral organs have found mitochondrial changes including disarrangement of cristae and a less dense matrix in the myocardium of rats exposed to MDMA and loud noise (Gesi et al., 2002). In isolated skeletal muscle mitochondria of MDMA-treated rats, there was an uncoupling of oxidative phosphorylation (Rusyniak et al., 2005). In liver mitochondrial fractions from rats, the activities of mitochondrial aldehyde dehydrogenase, 3-ketoacyl-CoA thiolases, and ATP synthase were significantly inhibited following MDMA exposure (Moon et al., 2008). It seems likely that MDMA actions in the mitochondria can go beyond inhibition of mitochondrial enzymes and uncoupling of oxidative phosphorylation, leading to mitochondrial ultrastructural changes.

Another interesting report, used mice deficient in a mitochondrial protein known as UCP-3 (for 'uncoupling protein-3'), and evaluated the thermogenic response to MDMA toxic doses (Mills et al., 2003). UCP-3 'knockout' mice, revealed a minor increase in body temperature following MDMA, when compared to wild type animals (Mills et al., 2003). Therefore, the rise in the skeletal muscle temperatures that is associated with MDMA administration requires the activity of this mitochondrial uncoupling protein-3.

It is worth to mention that the doses of MDMA used in most animal studies are high, and do not always correlate with human doses. In mice, it is estimated that a 25 % lethal dose to be about 50 mg/kg injected intraperitoneally (Fantegrossi et al., 2003), although this value might be slightly different according to the mouse strain. In fact, two studies used this dose by means of binge dosing scheme (Puerta et al., 2010; Barros-Miñones et al., 2015), and another one even exceeds it (Gesi et al., 2002). In rats, according to the allometric scaling principles, a dose of 40 mg/kg in adult rats, used in a daily session, is approximately equivalent to 700 mg in a human with 70 kg (Teixeira-Gomes et al., 2016). According to the European Union report MDMA pills range from 118 to 210 mg (EMCDDA, 2021), and therefore that dose could mean an average intake of 6 pills in a single session, a rather extreme scenario. This method of scaling does not account for the differences in MDMA metabolism or administration route between humans and rats, but is certainly of great value for an approximate extrapolation. Looking to the studies described in Table 2, six of them use the 40 mg/kg dose (Rusyniak et al., 2005; Alves et al., 2007; Alves et al., 2009a; Alves et al., 2009b; Taghizadeh et al., 2016; Taghizadeh et al., 2020a), and therefore caution must be applied when translating these results to humans. Once again, as mentioned previously, these studies are relevant from a mechanistic point of view and can steer future research using lower doses and more sensitive techniques.

## 4. Conclusion remarks

Overall, there is a consensus for MDMA toxic actions at the mitochondrial level. Despite the fact that establishing a direct translation to humans of the findings of these studies given the high doses and concentrations of MDMA used in some reports, they are very important from the mechanistic point of view. Given that the brain and liver are major target organs for MDMA toxicity, most reports focus on MDMA effects at the mitochondria of these organs. Such effects have been associated with deregulation on ATP levels, inhibition of mitochondrial complexes, collapse of  $\Delta\Psi_m$  and induction of mitochondrial permeability transition, and generation of oxidative stress, leading to mitochondrial DNA deletions. In addition, the deficits in mitochondrial axonal transport of mitochondria and increased fragmentation of axonal mitochondria were seen in neurons exposed to MDMA or its metabolites. Still, it remains to be established whether MDMA effects at the mitochondria results from a direct action of the drug

**Table 2**Main findings of studies conducted *in vivo* evaluating MDMA effects at the mitochondrial level. Studies are sorted by publication date.

Animal model	MDMA dose (administration route)	Main findings	Reference
Male Sprague Dawley rats (200 – 290 g)	MDMA (100 µM), MAL (100 mM) or the combination of both MDMA/MAL for 8 h (microdialysis probe into the striatum)	MDMA alone did not produce long-term depletion of brain DA or 5-HT, but MDMA combined with Malonate (MAL), a mitochondrial inhibitor that inhibits succinate dehydrogenase, evoked long-term depletion of both DA and 5-HT in the striatum brain tissue.	(Nixdorf et al., 2001)
Male C57 black mice (9–10 weeks old)	20 mg/kg × 3, every 2 h (intraperitoneal)	The myocardium of animals exposed to MDMA and loud noise (100dBa for 6 h) showed several tissue changes at the ultrastructural level, namely mitochondrial changes including disarrangement of cristae and a less dense matrix. These mitochondrial changes were not seen with MDMA alone.	(Gesí et al., 2002)
Swiss Black mice control and UCP-3 knockout	10–40 mg/kg (not described)	Mice deficient in mitochondrial protein UCP-3 (for ‘uncoupling protein-3’) have a diminished thermogenic response to the drug MDMA and survive to doses that are lethal to wild-type mice.	(Mills et al., 2003)
Male Sprague-Dawley rats (175 – 300 g)	40 mg/kg (subcutaneous)	Beta-ATP signal areas after MDMA treatment showed significant reductions (15 %) from the baseline values with corresponding increases in inorganic phosphate (88 % increases) and decreases in intracellular pH. In isolated skeletal muscle mitochondria of MDMA-treated rats there was an increase in complex 1-mediated respiratory control index without significant differences in complex 2. MDMA treated animals revealed uncoupling of oxidative phosphorylation in the skeletal muscle.	(Rusyniak et al., 2005)
Adolescent male Wistar rats (postnatal day 40)	10 mg/kg × 4, every 2 h (intraperitoneal)	Mitochondria isolated from several brain areas (prefrontal cortex, ventral mesencephalon, striatum, raphe nuclei, amygdala, and hippocampus) were analyzed and mitochondrial DNA revealed that NDI (nicotinamide adenine dinucleotide phosphate dehydrogenase subunit I) and NDII (nicotinamide adenine dinucleotide phosphate dehydrogenase subunit II) subunits of mitochondrial complex I and cytochrome c oxidase subunit I of complex IV suffered deletions, two weeks following MDMA exposure. Inhibition of monoamine oxidase type B (MAO-B) by selegiline (2 mg/kg intraperitoneal, 30 min before MDMA) avoided mitochondrial DNA deletions and oxidative stress.	(Alves et al., 2007)
Male Wistar rats (290 – 340 g)	5 mg/kg × 3, every 2 h (intraperitoneal)	Seven days following MDMA treatment rats revealed brain striatal 5-HT depletions, that were fully prevented by minoxidil (4 nmol microinjected into the striatum right before MDMA), via opening of a mitochondrial ATP-sensitive potassium channels.	(Goñi-Allo et al., 2008)
Male Sprague Dawley rats (225–250 g)	10 mg/kg × 2, 24hr apart (oral)	Mitochondrial fractions isolated from rat livers after the second dose revealed that MDMA increased oxidative stress, caused oxidative inactivation of several mitochondrial enzymes. Activities of mitochondrial aldehyde dehydrogenase, 3-ketoacyl-CoA thiolases, and ATP synthase were significantly inhibited following MDMA exposure.	(Moon et al., 2008)
Adolescent male Wistar rats (postnatal day 40)	10 mg/kg × 4, every 2 h (intraperitoneal)	Mitochondria isolated from several brain areas (prefrontal cortex, ventral mesencephalon, striatum, raphe nuclei, amygdala, and hippocampus) were analyzed and mitochondrial DNA revealed that NDI (nicotinamide adenine dinucleotide phosphate dehydrogenase subunit I) and NDII (nicotinamide adenine dinucleotide phosphate dehydrogenase subunit II) subunits of mitochondrial complex I and cytochrome c oxidase subunit I of complex IV suffered deletions, two weeks following MDMA exposure. Acetyl-L- carnitine (100 mg/kg intraperitoneal, 30 min before MDMA) prevented mitochondrial DNA deletions and brain serotonergic depletions.	(Alves et al., 2009a)
Adolescent male Wistar rats (postnatal day 40)	10 mg/kg × 4, every 2 h (intraperitoneal)	Inhibition of monoamine oxidase type A (MAO-A) by clorgyline (1 mg/kg intraperitoneal, 30 min before MDMA) had no protective effect on MDMA-induced alterations on brain mitochondria (increased lipid peroxidation, protein carbonylation and decrease in the expression of the respiratory chain subunits II of reduced nicotinamide adenine dinucleotide dehydrogenase and I of cytochrome oxidase).	(Alves et al., 2009b)
Male Wistar rats (290 – 340 g)	5 mg/kg × 3, every 2 h (intraperitoneal)	Oral administration of sildenafil citrate (1.5 or 8 mg/ kg, 30 min before MDMA) protected against MDMA-induced long-term reduction of brain serotonin by a mechanism involving mitochondrial ATP-sensitive potassium channel opening.	(Puerta et al., 2009)
Male Swiss-Webster mice (25 – 30 g)	10 + 20 + 30 mg/kg, every 2 h (intraperitoneal)	MDMA decreased mitochondrial complex I activity in the brain striatum of mice, which was evident 1 h after drug administration and remained significantly below control values for up to 24 h later. No changes were observed in the activity of any of the other mitochondrial complexes II, II/III, IV.	(Puerta et al., 2010)
Male C57BL/6J mice (25–30 g)	10 + 20 + 30 mg/kg, every 2 h (intraperitoneal)	MDMA caused a significant decrease in mitochondrial complex I activity in the brain striatum of animals 1 h after MDMA administration, an effect completely avoided by reserpine/alpha-methyl-p-tyrosine pretreatment (a drug combination that depletes both vesicular and cytoplasmic pools of dopamine 5 + 300 mg/kg intraperitoneal). Meanwhile, pretreatment with the dopamine uptake blocker GBR 12,909 (10 mg/kg intraperitoneal) or with the non-specific MAO inhibitor pargyline (15 mg/kg intraperitoneal), significantly attenuated complex I inhibition and dopamine depletion evoked by MDMA.	(Barros-Miñones et al., 2015)
Male Wistar rats (200 – 250 g)	5 or 10 or 15 mg/kg × 4, every 24 h (intraperitoneal)	Whole brain mitochondria isolated from the rats treated with MDMA (4 days after the first dose) showed a significant increase in ROS formation, collapse of ΔΨ <sub>m</sub> , mitochondrial swelling, and outer membrane damage, cytochrome c release and increased ADP/ATP ratio.	(Taghizadeh et al., 2016)

Table 2 (continued)

Animal model	MDMA dose (administration route)	Main findings	Reference
Adolescent male Wistar rats (postnatal day 40)	5 mg/kg × 3, every 2 h (intraperitoneal)	MDMA decreased ATP levels in the brain frontal cortex of adolescent rats, but not in other areas such as the hippocampus, cerebellum or striatum.	(Teixeira-Gomes et al., 2016)
Adolescent (postnatal day 40) and aged (mean 20 months old) male Wistar rats	Adolescent: 5 mg/kg × 3, every 2 h Aged: 5 mg/kg × 2, every 2 h (intraperitoneal)	A significant decrease on ATP levels in the brain cerebellum area of aged rats exposed to MDMA was found, but not in other areas such as the hippocampus, cortex or striatum. Regarding the adolescent group, no differences were found in any brain area.	(Feio-Azevedo et al., 2018)
Male Wistar rats (200 – 250 g)	5 or 10 or 15 mg/kg × 4, every 24 h (intraperitoneal)	MDMA-induced impairments to brain mitochondrial function were significantly correlated with the concentrations of both MDMA and its metabolite 3,4-Methylenedioxyamphetamine (MDA) in plasma, cerebrospinal fluid, and brain.	(Taghizadeh et al., 2020a)
Male Wistar rats (200 – 250 g)	0.5 or 1 µg (intra-hippocampal infusion)	Bucladesine (10 and 100 µM), a membrane-permeable analog of 3', 5'-cyclic adenosine monophosphate, 5 min after MDMA infusion, attenuated MDMA-induced increase in ROS formation, collapse of $\Delta\Psi_m$ , mitochondrial swelling, and outer membrane damage, cytochrome c release and increased ADP/ATP ratio in isolated brain hippocampal mitochondria.	(Taghizadeh et al., 2020b)

$\Delta\Psi_m$ , mitochondrial membrane potential.

and/or its metabolites or rather through indirect mechanisms, like hyperthermia and oxidative stress.

### CRedit authorship contribution statement

**João Paulo Capela:** Conceptualization, Writing – original draft.  
**Félix Dias Carvalho:** Writing – review & editing.

### 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 paper.

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