Pharmacology and neurotoxicity of 3,4-methylenedioxymethamphetamine (MDMA).

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Abstract

3,4-methylenedioxymethamphetamine (MDMA) is a psychostimulant drug that causes feelings of increased euphoria and love, increased sociability and increased energy. MDMA acts on the serotonin transporter (SERT), dopamine transporter (DAT), monoamine oxidase B, serotonin 2A and 2B receptor and tryptophan hydroxylase. The actions of MDMA on these proteins cause the desired effects of the drug by increasing the serotonin concentration in the synaptic cleft. Furthermore, MDMA increases the dopaminergic concentration in the synaptic cleft, which causes increased energy. MDMA is believed to cause neurotoxicity in a number of ways. During the metabolism of MDMA highly reactive *ortho*-quinones are formed, these can undergo redox cycling which may lead to the formation of free radicals. These free radicals promote alkylation of crucial proteins and/ or DNA, resulting in neuronal damage. Moreover, free radicals also interfere with the mitochondrial electron transport chain and cause a state of energy crisis in the neuron in this way. This mitochondrial dysfunction can cause serious problems in the functioning of the neuron, which may eventually lead to apoptosis. Besides the *ortho*-quinone metabolites of MDMA, monoamine metabolism and dopamine oxidation also contribute to oxidative stress that can lead to serotonergic terminal loss and apoptosis. Animal research has shown that MDMA is able to reduce SERT expression and protein levels, as well as decrease the availability of serotonin by its actions on the SERT and tryptophan hydroxylase. In addition, it has been found that MDMA-induced neurotoxicity is highly dependent on the serotonin 2A receptor activation. In human ecstasy users it was found that MDMA use leads to reduced SERT and $5HT_{2A}$ receptor densities throughout the brain. There are indications that these changes in SERT and $5HT_{2A}$ receptor densities may be reversible when MDMA use is stopped. However, results are not conclusive since conflicting results have been found. It has been found that MDMA use leads to impairments in verbal memory and prospective memory performance. Furthermore, cessation of ecstasy use may cause depression symptoms.

MDMA use is often combined with nicotine, cannabis or alcohol. It was found that when nicotine is combined MDMA, nicotine does not alter the neurotoxic effect of MDMA. Moreover, Δ9-tetrahydrocannabiol (THC) seems to be able to protect neurons from the toxic effect of MDMA. It is thought that this neuroprotective effect of THC is due to its antioxidant properties. Results and conclusions of studies investigating the neurotoxic effects of MDMA should be interpreted with care, since most of them have important limitations. Animal studies often have limitations to their translational value. Whereas human studies often use polydrug users instead of MDMA alone users. Future research to the neurotoxicity of MDMA is needed to address issues as why there are differences between men and women in susceptibility to neurotoxity of MDMA. Furthermore, it should be investigated whether the MDMA-induced changes in the serotonin system are reversible or not.

1. Introduction

3,4-methylenedioxymethamphetamine (MDMA) is the active ingredient in ecstasy tablets[1,2]. Ecstasy or MDMA use is popular with young adults, who often use it recreationally at clubs or "rave parties"[1–4]. MDMA is mostly taken because it causes feelings of euphoria and love, increased sociability, and increased energy in such a way that people are able to party all night[1,2,4,5]. MDMA users are often polydrug users, who also use other drugs and often combine ecstasy with alcohol, cigarettes or cannabis[4–6]. MDMA consumption has increased significantly over the last decades[1,2]. The European monitoring centre for drugs and drug addiction has estimated that in Europe approximately 1.3 million people in the age group 15-35 used ecstasy in 2012[7]. Furthermore, it was estimated that 3.1% of adults in Europe have used ecstasy in their lifetime[7].

MDMA causes an acute hyperthermic response, together with increased blood pressure and increased heart rate[8–11]. This combination of effects can be fatal[8–11]. Body temperature after MDMA use may rise to a temperature of 43 degrees Celsius[11]. This is especially dangerous because MDMA is often used at parties that involve crowded conditions and high ambient temperatures[10,11]. In addition, MDMA users are physically active. These conditions may increase body temperature of MDMA users even further, which will pose them at a great risk of dehydration[10]. Compensation of the hyperthermic response of MDMA by drinking large amounts of water is an indirect risk of ecstasy use, as many cases of water intoxication have been reported[12].

In this article the pharmacology and neurotoxic effects of MDMA are reviewed. Both preclinical and clinical research on the damaging effects of MDMA on the brain will be addressed. Additionally the effects of ecstasy or MDMA use on neuropsychologal measures will be described. Furthermore, the effects of combined use of MDMA with nicotine, THC and alcohol on neurotoxicity are discussed. Finally, the findings of all studies will be critically assessed and suggestions for future research will be discussed.

2. Pharmacology

MDMA administration results in acute release of serotonin, dopamine and norepinephrine in the brain as well as inhibition of the serotonin transporter (SERT), dopamine transporter (DAT) and norepinephrine transporter (NET) [1,8,13–16]. MDMA has the strongest effect on the release of serotonin[17]. Serotonin is involved in processes such as mood, cognition and memory in humans[17,18]. Many of the desirable effects of MDMA are caused by the release of serotonin that MDMA stimulates[1]. The increase in dopamine causes the stimulating effects of ecstasy[19].

MDMA is believed to increase the synaptic concentration of serotonin in three different ways[8,15,20]. First, MDMA is a substrate of the SERT and is able to use this transporter to enter the neuronal terminal[21]. At high concentrations MDMA is also able to enter via diffusion. Inside the neuron MDMA stimulates the release of serotonin from storage vesicles[15]. *In vitro* it was found that MDMA enters the vesicles using vesicular monoamine transporter (VMAT)[22]. In this way, it may also decrease VMAT protein expression, since reductions in VMAT-2 protein expression have been found in striatal tissue of 10-week old mice given MDMA binge administration[23]. Second, MDMA causes increased synaptic serotonin levels by inhibiting monoamino oxidase B (MAO-B) in humans[14]. MAO-B is responsible for the degradation of serotonin inside the neuron[14,15].

MDMA partly inhibits MAO-B, which causes serotonin levels to remain high inside the serotonergic neurons [8,15]. Third, both *in vivo* and *in vitro* research has shown that MDMA activates synaptic $5HT_{2B}$ receptors directly[20], which may result in increased serotonin levels in the nucleus accumbens and ventral tegmental[20,24].

Furthermore, it has been shown in rats that MDMA metabolites also inhibit tryptophan hydroxylase, the rate limiting enzyme in the synthesis of serotonin[23]. The acute increase of these neurotransmitter levels is followed by a period of depletion afterwards, because of this inhibition[8].

MDMA also significantly increases synaptic dopaminergic levels, by inhibiting the DAT[21]. Increases in dopaminergic activity in the nigrostriatal pathway, are responsible for the stimulating effect of MDMA[19]. Furthermore, the inhibition of DAT also causes increased dopamine levels in the nucleus accumbens[2,25]. This is thought to cause the euphoric effect of MDMA[25]. Furthermore MDMA causes more dopamine release than serotonin release in the nucleus accumbens, striatum and caudate[26]. MDMAs effect on dopaminergic neurons is regulated by acting on both D1 and D2 receptors[27,28]. Most drugs that increase dopaminergic activity in the nucleus accumbens have rewarding effects that cause them to be addictive[25]. This is the case for psychostimulant drugs of abuse such as cocaine and amphetamine[2,25,29]. As for MDMA, findings about the rewarding effect of the drug are inconsistent and seem to be dependent on the dosage used[2]. Brennan *et al* found that activation of D1 and D2 receptors maintain self-administration of MDMA in rats[27]. Furthermore, it was shown that D1 and D2 antagonists attenuate MDMA seeking behaviour in rats[27,28,30]. This shows that MDMA causes drug-seeking behaviour by acting on both D1 and D2 receptors. On the other hand, alternative studies found that MDMA does not act rewarding[31,32]. Multiple studies have shown that only high doses of MDMA may cause rewarding effects[33–35].

Compared to other psychoactive substances such as cocaine and D-amphetamine MDMA is less addictive[24]. This may be explained by the fact that MDMA is more potent in releasing serotonin than dopamine. MDMA inhibits SERT three times more than DAT, and MDMA causes 1.5 times more serotonin release than dopamine release *in vitro* and *in vivo*[24,29,36].

MDMA is mainly metabolised in the liver and follows two major pathways[14,15,36–38]. MDMA is first metabolised in the liver where it is metabolised to 3,4-methylenediozyamphetamin (MDA)[15,38]. MDMA and MDA are then further metabolised to form N-methyl-α–methyldopamine (N-Me-α-MeDA) and α-methyldopamine (α-MeDA)[15,38]. Catechol-O-methyltransferase (COMT) can interact with both N-Me-α-MeDA and α-MeDA, which results in the formation of 3,4-dihydroxymethamphetamine (HHMA) and 3,4-hydroxyamphetamine (HHA)[15,36,38]. HHMA and HHA are further processed to form 4-hydroxy-3-methoxymethamphetamine (HMMA) and 4-hydroxy-3-methoxyamphetmaine (HMA)[13–15,36,38]. HHMA and HHA can generate highly reactive *ortho*-quinones by undergoing oxidation[13,15,36–38]. These quinones can undergo redox cycling, this is a repetitive coupled reduction and oxidation reaction process where electrons are transferred that can cause the formation of free radicals. During the redox cycling of the *ortho*-quinones reactive oxygen species (ROS) and reactive nitrogen species (RNS) will be formed[13,15,36,38]. The ortho-quinones are also able to form adducts with glutathione (GSH) and other thiol-containing compounds[13,15,36,38]. The GSH conjugates remain active and are capable of undergoing the addition of a second GSH molecule, which will result in a 2,5-bis-glutathionyl conjugate. Via the mercapturic acid

pathway these GSH-conjugated metabolites are taken up in the brain through the L-transporter for neutral amino acids located in the capillaries of the blood brain barrier[13,15,36,38]. In the brain they can be further metabolised to N-acetylcysteine (NAC) conjugates[13,15,36,38]. See figure 1 for a more detailed overview of the metabolism of MDMA.

In summary, MDMA increases synaptic concentrations of serotonin, dopamine and norepiniphrine. MDMA blocks the serotonin and dopamine reuptake by SERT and DAT, inhibits the serotonin degradation by MAO-B, and directly activates the $5HT_{2B}$ receptor, which causes serotonin and dopamine concentration to increase in the synapse. Furthermore, MDMA inhibits tryptophan hydroxylase, which causes serotonin depletion. In addition during the metabolism of MDMA highly reactive *ortho*-quinones are generated. These quinones can undergo redox cycling in which electrons are transferred, which will result in the formation of free radicals ROS and RNS because.

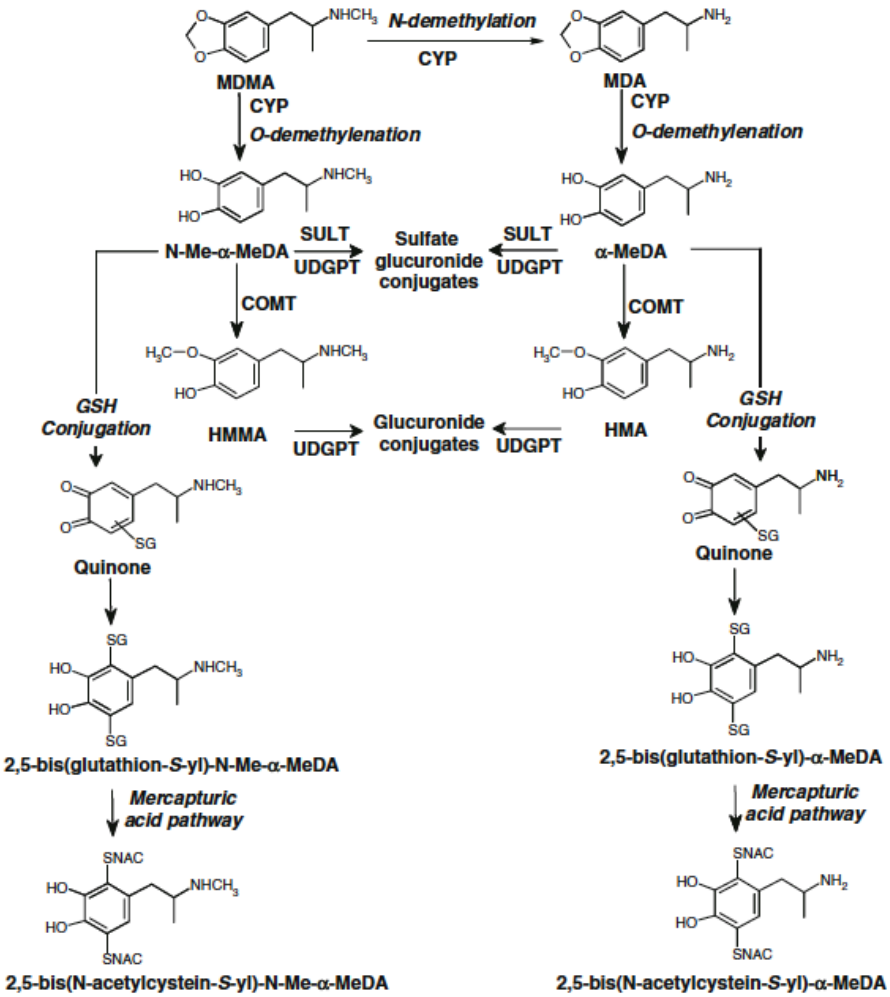

Figure 1: Schematic representation of the metabolism of MDMA[15].

3. Neurotoxicity

Neurotoxicity can be defined as a process that occurs when normal processes in the nervous system are altered in such a way that it damages or may even kill neurons. Loss of neurons or loss of function of neurons can result in a decrease of certain brain

functions. Substances that cause neurotoxicity are called neurotoxins. Neurotoxicity induced by MDMA has been studied from different perspectives.

3.1. Preclinical research

In a study in rats, MDMA has been found to first increase SERT mRNA expression during the first 7 days after administration, decrease SERT expression after 21 days and recovery of SERT expression occurs 180 days after a single dose of MDMA[39]. In this study fibre density in the hippocampus was most affected. In this region a decrease in fibre density was still visible 180 days after administration of MDMA[39]. In another study MDMA was binge administered and after 2 weeks, a ~50 fold reduction in SERT gene expression and ~10 fold reduction in VMAT-2 gene expression was observed[23]. The ~50 fold decrease in SERT gene expression resulted in a 50% decrease in SERT protein levels in the synapses[23]. As mentioned before MDMA inhibits tryptophan hydroxylase[15,23,36]. This causes a depletion of serotonin after MDMA use[15,23,36]. Thus, MDMA is able to reduce SERT expression and protein levels, as well as decrease the availability of serotonin.

Interestingly it has been found that certain metabolites of MDMA are more neurotoxic for rats than MDMA itself[13]. Moreover, a direct MDMA, MDA, HMMA and HHMA injection in the rat brain does not cause neurotoxicity[38]. Whereas α-MeDA, N-me-α-MeDA, and the GSH and NAC conjugates did show neurotoxic effects *in vtiro*[13,38,40]. These metabolites contributed to the formation of ROS in serotonergic cells and serotonergic terminals[13,38,40]. These free radicals can cause axonal loss or even neuronal death[11,38,41].

The GSH and NAC conjugates of MDMA can specifically enter serotonergic neurons via the SERT[15]. Once inside they will undergo redox cycling, which will cause the formation of ROS and RNS[13,38]. Furthermore, they will promote alkylation of crucial proteins and/ or DNA leading to cell damage[15,36,38]. GSH conjugates can be eliminated from the brain much faster than NAC conjugates, which are able to accumulate in the brain[15]. This further increases the toxic potential of these NAC conjugates[15]. Production of ROS and RNS are important contributors to MDMA induced neurotoxicity[11,38,40]. ROS and RNS can block the electron flow from nicotinamide adenine dinucleotide + hydrogen (NADH) dehydrogenase to coenzyme Q in rats[42]. This will result in altered function of proteins of the mitochondrial electron transport chain, which will cause a state of energy crisis in these neurons[42]. This mitochondrial dysfunction can cause serious problems in the functioning of the neuron, which may eventually lead to apoptosis[42].

MDMA induced neurotoxicity in rats is highly dependent on $5HT_{2A}$ activation[11]. Activation of $5HT_{2A}$ receptors causes an increase in intracellular calcium levels[15]. This increase will lead to an overproduction of nitric oxide (NO), and subsequently the production of the free radicals ROS and RNS[15]. ROS and RNS can cause oxidative stress that leads to apoptosis of the neurons accompanied with caspase 3 activation[11]. Caspase 3 belongs to a family of cysteine proteases that play an essential role in apoptosis[43]. *In vitro* research has shown that Cortical neurons expressing the $5HT_{2A}$ receptor are highly susceptible to MDMA-induced toxicity[11,41]. This MDMA induced neurotoxicity results in dendrite and axonal loss as well as apoptosis[11,41]. Antagonists of the $5HT_{2A}$ receptor are also able to reduce the depletion of GSH levels that MDMA causes[11]. These findings all indicate the importance of the $5HT_{2A}$ receptor in mediating MDMA induced neurotoxicity.

Besides the MDMA metabolites, monoamine metabolism and dopamine oxidation also contribute to oxidative stress that can lead to serotonergic terminal loss and apoptosis[15,40]. Dopamine release caused by MDMA is stimulated by post-synaptic serotonin receptors after the acute serotonin burst is released in mice[20,44]. It is hypothesised that the dopamine that is released is transported into the serotonin terminal and is deaminated here by MAO-B[15,18,44]. In this process RNS and ROS are formed[18,40,44]. It is thought that tryptophan hydroxylase may be oxidized by these reactive species[44]. Because ROS and RNS are so reactive it is thought that they react before they can reach the cell body and therefore mainly the neuron terminals mainly damaged[44]. Furthermore, a clinical study by Schilt *et al* found that extracellular dopamine is metabolised by COMT[18]. Since COMT is an import enzyme in the metabolism of MDMA, a decrease in COMT function will increase serotonergic damage in two ways.

MDMA causes a rapid increase in interleukin 1β (IL-1β) in the hypothalamus and cortex of rats[45]. MDMA activates microglia cells in the hippocampus, where they cause an inflammatory response[45,46]. Both the increase in IL-1β and the increase in microglia activity cause a disruption in blood brain integrity and neuronal degeneration[45,46].

Furthermore, hyperthermia was found to further enhance neurotoxicity *in vivo* and *in vitro*[15,38,41,47]. MDMA increases body temperature by activating serotonin receptors in the preoptic nucleus of the hypothalamus[48]. High levels of extracellular dopamine also contribute to hyperthermia in humans[18]. It has been suggested that hyperthermia may increase neurotoxicity by altering transport function or altering MDMA metabolism[47].

Taken together these studies show that ROS and RNS may cause a state of energy crisis in the neuron that can lead to apoptosis[42]. Besides the MDMA metabolites, monoamine metabolism and dopamine oxidation in rats also contribute to oxidative stress that can lead to serotonergic terminal loss and apoptosis[15,40]. Furthermore, MDMA has been found to reduce SERT expression and protein levels. MDMA also decreases the availability of serotonin by blocking the SERT and inhibiting tryptophan hydroxylase. In addition, it was found that MDMA induced neurotoxicity is highly dependent on $5HT_{2A}$ activation and MDMA causes hyperthermia, which aggravates the neurotoxic effects of MDMA[11].

3.2. Neuroimaging research

Much research has been done on the effect of MDMA in humans. Most studies are conducted using neuroimaging with people who are either current or abstinent MDMA users[49–52]. Most neuroimaging techniques investigating the neurotoxic effects of MDMA use positron emission tomography (PET) or single photon emission computed tomography (SPECT) in combination with radioactive tracers[49–52]. The radioactive tracer emits gamma rays that can be visualised with a PET or SPECT scan. SPECT tracers are longer lasting than those of PET, but the resolution of SPECT is poorer. Furthermore, functional magnetic resonance imaging (fMRI) is used to measure brain activity patterns during the performance of cognitive tasks.

Several studies have shown that MDMA use results in reduced SERT densities in serotonergic neurons throughout the brain[52–55]. In a PET study with recreational ecstasy users, a marked decrease in SERT binding is demonstrated in the cerebral cortex and hippocampus, but not in the SERT-rich striatum[55]. This indicates that the reduced SERT binding in the brain was region-specific[55]. In heavy ecstasy users, a dose-dependent

decrease in SERT densities was shown in subcortical regions such as the putamen and the midbrain [50,54]. In a SPECT study by Klomp *et al* it was found that MDMA has a different effect on the developing brain than it has on the adult brain[56]. It was observed that people that started using MDMA at an early age (<18 years) had a less pronounced decrease in SERT densities as compared to people who started using MDMA at a later age (≥18 years)[56]. This might be because the degree of plasticity in a developing brain is higher than at later stages[56]. Because of this, MDMA seems to be less toxic to the developing brain[56]. Interestingly, reduced SERT densities were correlated with impaired prospective memory[57]. In abstinent MDMA users, it was found that people who remained abstinent form MDMA longer had better SERT binding capacities[50]. There are indications that the reduced availability of SERT might be reversible when MDMA use is stopped, but results are not conclusive[17,50,58,59]. Di Iorio *et al* found that persistent changes in SERT and $5HT_{2A}$ receptor availability were present in 2 weeks abstinent MDMA users in a PET study[58]. Furthermore, McCann *et al* found reduced SERT levels in 2 weeks abstinent MDMA users in a quantitative PET study[50]. Furthermore, Thomasius *et al* performed a PET study and found that changes in SERT availability due to heavy MDMA use were reversible if MDMA use was stopped for at least 9 months[53].

PET studies that measured cerebral metabolism of glucose showed that in recreational ecstasy users, hypometabolism of glucose in the frontal cortex and hippocampus was correlated with deficits in verbal memory, while decreases in dorsolateral, prefrontal and parietal areas of the brain were correlated with verbal memory deficits in abstinent MDMA users[60]. A dose dependent effect is present between ecstasy use and prospective memory performance[57]. MDMA has also been found to reduce $5HT_{2A}$ receptor levels, while these receptors were upregulated when MDMA use was stopped[58]. Cowan *et al* concluded that MDMA use causes a reduction in $5HT_{2A}$ receptors and an increase of $5HT_{2A}$ receptor levels after MDMA use was stopped for at least 2 months[61]. $5HT_{2A}$ receptors were more present in MDMA users when MDMA had been used more intensely[58].

Ramaekers *et al* showed in an event related fMRI study that MDMA use causes an acute decrease of prospective memory performance in recreational ecstasy users[62]. However, Becker *et al* found long-term recovery of MDMA-induced serotonergic damage in a prospective fMRI study with participants who quit MDMA use themselves as compared to participants who continued using MDMA[59]. A decreased hippocampus activity was also observed in heavy and sporadic MDMA users in this study[59] De Win *et al* and Jager *et al* have found that a low dose of ecstasy in first time users does not lead to chronic neuronal damage[63,64]. Furthermore, it was found that one time ecstasy use caused no sustained effect on attention or memory[59]. It was also found that after incidental ecstasy use there were no changes in brain activity in the areas responsible for attention and memory[64]. This suggests that multiple doses of MDMA are needed to induce chronic neuronal changes. Furthermore, another study investigating heavy and moderate MDMA users, as well as ecstasy naïve drug using controls ruled out that a genetic predisposition was responsible for low SERT densities[65].

Altogether, neuroimaging studies in human ecstasy users have found that MDMA use leads to reduced SERT and $5HT_{2A}$ receptor densities throughout the brain. There are indications that these changes in SERT and $5HT_{2A}$ receptor densities may be reversible when MDMA use is stopped. Furthermore, it was found that incidental ecstasy use does

not cause sustained changes in attention or memory, nor does it cause changes in the brain areas responsible for these behaviours.

3.3. Neuropsychology

MDMA primarily affects serotonin neurotransmission in humans. Serotonin plays an important role neurocognitive functions such as attention, learning and memory, as well as in mood regulation[66]. Therefore, these processes could be affected in MDMA users. Multiple studies found that ecstasy use is associated with a decrease in verbal memory function[17,59,66]. The thalamus has an important function in neurocognitive processes and it is hypothesised that neuronal damage in this area is responsible for the decreased verbal memory performance[17]. In a study by Reneman *et al* it was found that heavy MDMA users which were abstinent for 1 year still showed impaired verbal memory function[65]. Another aspect of memory that is affected by ecstasy use is prospective memory, which can be explained as remembering to perform intended actions in the future[57,62]. Gallagher *et al* found that the average consumed dose of ecstasy per session is associated with the decrease in prospective memory performance[57].

Besides memory impairments, weight loss, anxiety and depression are also common problems with MDMA users[67,68]. These effects appear to be long-lasting, as de Win *et al* found that 12 months abstinent former heavy ecstasy users showed more depression symptoms than drug users that did not use ecstasy[49]. While it was found that symptoms of depression were related to the total number of ecstasy tablets used, there was no correlation found between depressive symptoms and serotonin deficits in a SPECT study by de Win *et al*[49]. Finally, in a prospective study, MDMA users scored lower on depression tests after first time ecstasy use than before[63].

MDMA has been found to not affect attention in novice, moderate and heavy ecstasy users[64,65,69]. Furthermore, Roberts *et al* found that ecstasy does not cause an attentional bias for ecstasy-related cues in heavy ecstasy users[70].

In conclusion, it has been found that MDMA use leads to impairments in verbal memory and prospective memory performance. Furthermore, cessation of ecstasy use may cause depressive symptoms. Long-term MDMA use does not seem to influence attention.

4. Interactions

Many ecstasy users use MDMA in combination with other psychoactive substances, such as nicotine, cannabis and alcohol[4,6]. Therefore, it is of high significance to determine how MDMA interacts with these substances. The effects of the combined use of MDMA with nicotine, cannabis and alcohol on neurotoxicity will now be discussed.

4.1. Nicotine

Nicotine acts on nicotinic acetylcholine receptors (nAChRs) located on dopaminergic neurons, and influences their mode and frequency of firing[71,72]. By activating nAChRs in the mesolimbic system (nucleus accumbens, VTA, striatum), the reward pathway is stimulated, which makes smoking cigarettes addictive[71,73–75]. MDMA can interact with certain nAChRs, of which the heterodimer $(\alpha4)_2(\beta2)_3$ and α7 homodimer are the most important[71–77]. MDMA antagonises the α4β2 nAChR and agonises the α7 nAChR[71–77]. When a nAChR is activated, depolarization occurs, which makes the neuronal membrane permeable for Ca^{2+} ions[71].

The increase in CA^{2+} influx is thought to activate calpain and caspase 3 pathways, which are involved in neurotoxicity[77]. Furthermore, increased Ca^{2+} concentrations may result in inhibition of DAT and thereby in enhanced dopamine levels in serotonergic neurons after MDMA use, this may lead to increased ROS concentrations[71,76,77]. ROS concentrations are increased because dopamine will accumulate in the neuron and will be degraded by MAO-B, which produces ROS as explained before in *chapter 3.1*[18,40,44,76]. This theory is supported by studies from Chipana *et al* and Ciduad Roberts *et al* who showed that MDMA has practically the same affinity for the α7 nAChR as for the SERT and that an α7 nAChR antagonist can prevent MDMA induced neurotoxicity[71,74,76]. Therefore it can be concluded that activation of the α7 nAChR plays a major role in MDMA induced neurotoxicity[71].

However, the combination of sustained nicotine use and MDMA use does not differ from MDMA use alone in increasing basal Ca^{2+} levels[77]. Therefore it is possible that neurons are able to react to sustained activation by nicotine in such a way that they return to normal Ca^{2+} levels[77].

Activation of the α4β2 nAChR seems to have a neuroprotective function *in vivo* and *in vitro*[78]. However, since MDMA antagonises the α4β2 nAChR, this neuroprotective effect is inhibited[77,78]. Therefore, nicotine is not able to protect neurons from MDMA induced neurotoxicity. Moreover, Pubill *et al* found *in vivo* that the combination of nicotine and MDMA use can have a synergistic effect in increasing heterodimer receptor levels in the cortex and α7 homodimer receptors in the hippocampus[75]. This could even enhance the neurotoxicity of MDMA[75]. Since both nicotine and MDMA have a higher affinity for the heterodimer receptor, the effect on this receptor is greater than on the α7 receptor[75].

Altogether, it is highly unlikely that nicotine is able to protect neurons from MDMA-induced neurotoxicity. If anything, it may augment toxicity associated with ecstasy use.

4.2. Cannabis

Cannabis is the most commonly co-used illicit drug with ecstasy34,79,80. The active ingredient in cannabis is Δ9-tetrahydrocannabiol (THC)79–81. The psychoactive effects of THC are mainly due to its agonistic actions on the cannabinoid receptor 1 (CB1)48,82.

Many acute effects of MDMA such as locomotor activity, body temperature, anxiety, mood and reward are modulated by the CB_1 receptor[48,83]. It is thought that THC is able to provide protection for the oxidative effects of MDMA due to its antioxidant properties. THC is able to provide protection for the oxidative effects of MDMA that create ROS in mice and rats[71,80]. The protective effect of THC is thought to be independent on CB_1 activation, but is more likely caused by unspecific antioxidant properties of THC[71,80,84]. Furthermore, THC causes hypothermia by activating CB_1 receptors in the preoptic nucleus of the hypothalamus[48,81]. It has been found that coadministration of THC with MDMA is able to prevent MDMA-induced hyperthermia in mice and rats[80,81]. Morley et al found that a selective CB_1 antagonist was able to reduce body temperature, but did not protect against serotonin depletion in rats[80]. In addition, Chipana et al showed in vitro that the antioxidant properties of THC are responsible for the neuroprotective effect[71]. Therefore it is suggested that the antioxidant properties of THC are able to block the free radical formation caused by MDMA[71,80]. Contradicting results to this theory have also been found[81]. Touriño et al found that the

neuroprotective effects of THC were dependent on inducing CB_1 mediated hypothermia using CB_1 and CB_2 deficient mice[81]. However, a study with humans found that co-administration of cannabis and MDMA does not reduce hyperthermia[10]. Moreover, this study found that MDMA and cannabis co-use in humans causes an increased body temperature comparable to the effect of MDMA alone[10]. The combination of the two substances only delayed the onset and increased the duration of the hyperthermia[10].

In summary, THC appears to be able to provide protection for the oxidative effects of MDMA that create ROS in mice and rats. Although THC does prevent MDMA-induced hyperthermia in animals, it is unclear how this is involved in its neuroprotective effects.

4.3. Alcohol

Alcoholic drinks are very popular and are regularly consumed at social events or parties[85]. MDMA is often used in combination with alcoholic beverages[85–87]. One of the reasons MDMA and alcohol are co-used is that alcohol can cause the euphoric effects of MDMA to last longer[88].

Combined ethanol and MDMA administration to rats has been found to cause long-term alterations in working memory performance and spatial orientation when measured with a radial arm maze[86]. These are thought to be the result of an increased neuronal loss and accumulation of microglia in the dentate gyrus region of the hippocampus as compared to MDMA treatment alone, since the integrity of the mature granule cells in the dentate gyrus was specifically damaged by the combination of alcohol and MDMA [86].

In humans it was demonstrated that alcohol administration alone did not affect body temperature, while the combined use of alcohol and MDMA prevented MDMA induced hyperthermia[85]. In addition, it was found that MDMA and alcohol co-use in rats leads to a decrease in serotonin concentration and SERT density in both cortex and hippocampus, accompanied by enhanced ROS formation. It has also been found that high doses of alcohol boost the neurotoxic effects of MDMA by enhancing ROS formation in rats[89]. Furthermore, it was found that MDMA and alcohol co-use in rats leads to a decrease in serotonin concentration and SERT density in both cortex and hippocampus.

Taken together, combined alcohol and MDMA administration to rats has been found to cause impairments in both working memory performance and spatial orientation, as well as ROS-induced reductions in serotonin levels and SERT density[86]. Combined use of alcohol and MDMA can reduce the MDMA induced hyperthermia in humans. It has also been found that high doses of alcohol boost the neurotoxic effects of MDMA by enhancing ROS formation in rats[89].

5. Discussion

This review discusses current literature regarding the neurotoxicity of MDMA. In addition, the effect of the combined use of MDMA with nicotine, THC or alcohol is described.

MDMA increases the synaptic concentration of the neurotransmitters serotonin, dopamine and norepinephrine in the brain. MDMA does this by blocking the SERT and DAT and activating the $5HT_{2B}$ receptor, which causes increased synaptic concentrations of dopamine and especially serotonin. Furthermore, MDMA inhibits MAO-B and tryptophan hydroxylase, which causes increased oxidation and decreased serotonin

availability in serotonergic neurons. It was shown that during the metabolism of MDMA highly reactive *ortho*-quinones are produced. These quinones can undergo redox cycling, which will result in the development of free radicals. These free radicals are able to cause a state of energy crisis that leads to apoptosis in the neuron[42]. Besides this, MDMA blocks the SERT and inhibits tryptophan hydroxylase, which reduces the protein levels of both SERT and serotonin. In addition, it has been found that MDMA induced neurotoxicity is highly dependent on MDMA-induced $5HT_{2A}$ activation. Neuroimaging studies in human ecstasy users have found that the use of MDMA causes reduced SERT and $5HT_{2A}$ receptor densities throughout the brain. This decrease in SERT and $5HT_{2A}$ receptor densities may be reversible when MDMA use is stopped for a long time. However, opposing results have also been found concerning the reversibility of MDMA-induced neurotoxicity, showing that results are not conclusive. The most seen cognitive deficits of ecstasy users are impairments in verbal memory and prospective memory performance. In addition, long ecstasy abstinence of cessation of ecstasy use may result in depression symptoms.

Ecstasy is often used in combination with the psychoactive substances nicotine, THC or alcohol, which could influence MDMAs neurotoxic effect. Nicotine has not been found to act neuroprotective. On the other hand, THC has been found to be able to provide protection for the oxidative effects of MDMA. Whereas THC seems to act hypothermic in animals it does not seem to do this in humans. Combined use of alcohol and MDMA can reduce the MDMA induced hyperthermia in humans. It has also been found that alcohol may boost the neurotoxic effects of MDMA by enhancing ROS formation in rats.

Research has shown that cannabis use may serve neuroprotective, it is however questionable how applicable this is to recreational cannabis users. The finding that cannabis may have neuroprotective properties may cause MDMA users to increase their cannabis use. This neuroprotective effect of THC should be put in perspective because of the following reasons. First, the found neuroprotective effect of cannabis is only found in doses much higher than recreational users normally use, it is therefore unlikely that a recreational THC dose would function neuroprotective[80]. Second, the found neuroprotective effect is a partial effect that only attenuates the serotonin depleting effects of MDMA[80,84]. Third, a study investigating the effects of chronic MDMA and cannabis use in rats found that cannabis was only neuroprotective the first day of injection[5].

Although alcohol consumption can enhance ROS formation caused by MDMA use, alcohol may protect against hyponatraemia caused by MDMA. Hyponatraemia is defined as a low sodium concentration in the blood, and can be considered as a potential fatal side effect of MDMA[85]. MDMA has been shown to induce hyponatraemia by promoting the production of ADH, which causes water retention and dilation of blood vessels[90]. Alcohol has been found to attenuate the MDMA induced increase in ADH[85]. Alcohol also promotes diuresis and in this manner changes the hydration regulation in the body[85].

It has been found that nicotine, cannabis or alcohol use may cause an individual to be more sensitive to the addictive effects of MDMA. The nAChR is thought to be involved in this increase in sensitivity towards the addictive effects of MDMA. Chronic nicotine exposure can cause upregulation of nAChRs, which has been linked to the addictive effects of MDMA[74,75]. In rats it was observed that pre-treatment with nicotine increased the motivation for MDMA[74]. Therefore, it is possible that smoking cigarettes will make someone more sensitive to the addictive effects of MDMA[75]. In addition, increasing

evidence suggests that the CB_1 receptor plays an important role in the rewarding effects of MDMA[34,48]. Robledo *et al* has shown that THC makes mice more sensitive to the reinforcing effects of MDMA[33]. MDMA preadministration is able to mediate the increase of dopamine in the nucleus accumbens that THC intake causes[33]. This mediating effect of MDMA is dependent on the fact whether or not MDMA is already present before THC is taken[33]. On the other hand this study also shows that the combination of THC and MDMA causes more dopamine outflow in the nucleus accumbens than MDMA alone [33]. Therefore it could be argued that THC increases the sensitivity to the addictive effects of MDMA. Finally, animal research using the conditioned place preference test has shown that alcohol use may increase the risk of compulsive MDMA use[31].

Many preclinical and clinical studies are subject to a number of limitations. Preclinical research that investigates the effects of MDMA is mostly performed in mice and rats. It is questionable how representative these animal studies are for the human situation. Rats show damage to serotonergic neurons after MDMA administration. In mice, on the other hand, MDMA acts as a dopaminergic neurotoxin[47,76]. Mueller *et al* suggested that pharmacodynamic factors may cause this difference in neurotoxic profile of MDMA between rats and mice[47]. This causes animal studies using mice to have low construct validity and makes their results less reliable. Furthermore, in rat studies a decrease in social interaction is observed, which is not comparable to the human situation[78,79]. For many people an increase in social interactions is one of the main reasons to take the drug[1,80,84]. Therefore, the rat model is also lacking some translational value. However, animal studies are crucial in MDMA research investigating mechanisms of MDMA-induced neurotoxicity. Furthermore, human research is bound by ethical considerations that make it impossible to test high dosages or induce MDMA-induced neurotoxicity in humans. In addition, animal studies allow for more control over drug intake, whereas this often cannot be controlled in human studies.

A much seen limitation in neuroimaging studies that investigate the neurotoxic effects of MDMA is that subjects are frequently polydrug users[4]. This makes it harder to draw conclusions about the specific effects of MDMA. As shown in this review, co-use of MDMA with other substances can influence MDMAs neurotoxicity. In future studies it may be advisable to include a control group of ecstasy naïve polydrug users as has been done by de Win *et al*[49]. Furthermore, in most neuroimaging studies participants have used MDMA, or still use MDMA[50–52]. It is not possible to exclude pre-existing differences between MDMA and control groups because baseline data are not present in this model. For this reason prospective studies with ecstasy naïve subjects have been performed[63,66]. In these studies participants were included in these study based on the fact that they ecstasy naïve but considered using ecstasy in the future[63,66]. Such a design may be preferable because it will yield better control subjects who are more comparable to the ecstasy group. Another limitation to human ecstasy research is that it is difficult to find out how much MDMA an individual exactly used. Pill contents may vary between batches and ecstasy users do not always test their ecstasy pills. In addition to this, pills sold as ecstasy may also contain other psychoactive substances such as amphetamines[6]. Therefore two individuals that have used the same amount of pills may have consumed a totally different amount of MDMA. This could influence clinical research, as for instance mentioned by Jager *et al*, who suggested that the reduced memory performance that is often observed in MDMA users may in fact be attributable to amphetamine use[69].

Suggestions can be made for future research on the neurotoxic effects of MDMA. It may be interesting to further investigate the difference in susceptibility to the neurotoxic effects of MDMA between male and female users. In a previous study it has been found that female MDMA users are more susceptible to the neurotoxic effects of MDMA than male users[91]. Furthermore, it was found that women have reduced $5HT_{2A}$ receptor binding levels as compared to men[58]. In animal research it was found that control females have higher SERT densities than control males [83]. Therefore, it would be interesting to investigate this sex difference further and try to find out what exactly causes women to be more susceptible to the neurotoxic effects of MDMA. In addition to this, it is important to know whether reductions in SERT and $5HT_{2A}$ densities caused by MDMA are reversible when MDMA use is stopped. More research should focus on this subject. As for future research investigating interactions of MDMA with other psychoactive substances, the interaction with cannabis seems to be the most interesting one to investigate as THC is thought to possess antioxidant properties that block free radical formation. Studies that show the neuroprotective effect of THC are mostly based on effects observed in adult animals rather than adolescent animals[80,81,83]. However, cannabis and MDMA are co-used the most during the adolescent life stage[5,7]. Therefore, the combined effect of these drugs on adolescents is of particular interest. Especially since CB_1 receptor levels are the highest during adolescence. Moreover, chronic CB_1 activation during adolescence can have cognitive impairments and emotional alterations in adulthood[5].

In conclusion, findings of studies on the neurotoxic effects of MDMA discussed in this review suggest that MDMA cause neurotoxicity via multiple pathways. In the metabolism of MDMA highly reactive *ortho*-quinones are formed that can undergo redox cycling and form free radicals in this way. Furthermore, MDMA-induced dopamine oxidation and monoamine metabolism cause neuronal damage. Neuroimaging studies in human ecstasy users have found reduced SERT and $5HT_{2A}$ receptor densities throughout the brain. MDMA is often combined with other psychoactive substances that may influence neurotoxicity. Combining nicotine with MDMA does not seem to increase the MDMA-induced neurotoxicity, while combining alcohol consumption and MDMA use may boost the neurotoxic effects of MDMA. Interestingly, when cannabis use is combined with MDMA use, the antioxidant properties of high concentrations of THC seem to protect against the neurotoxic effects of MDMA. It is important that these conclusions are interpreted with care, because of limitations to translational value of animal studies and methodological issues associated with retrospective research in ecstasy users. Future studies should focus on a possible sex difference in the susceptibility to the neurotoxic effects of MDMA and the reversibility of MDMA-induced changes in SERT densities after MDMA use is stopped.

6. References

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