Chapter 38

# MDMA and Glutamate

## Implications for Hippocampal Neurotoxicity

John H. Anneken[1], Stuart A. Collins[2], Bryan K. Yamamoto[2], Gary A. Gudelsky[3]

*[1]Department of Psychiatry, Wayne State University, Detroit, MI, USA; [2]Department of Neurosciences, University of Toledo College of Medicine, Toledo, OH, USA; [3]James Winkle College of Pharmacy, University of Cincinnati, Cincinnati, OH, USA*

### Abbreviations

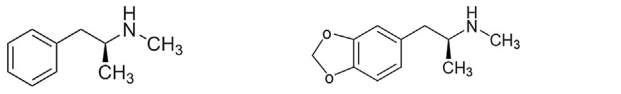
**5-HIAA** 5-Hydroxyindoleacetic acid
**5-HT** 5-Hydroxytryptamine (serotonin)
**COX** Cyclooxygenase
**DAT** Dopamine transporter
**FLX** Fluoxetine
**GABA** Gamma-aminobutyric acid
**GAD67** Glutamate decarboxylase 67
**GFAP** Glial fibrillary acidic protein
**METH** Methamphetamine
**MDMA** 3,4-Methylenedioxymethamphetamine
**SERT** Serotonin transporter
**SSRI** Selective serotonin reuptake inhibitor
$\mathbf{X_c^-}$ Cystine–glutamate antiporter

## INTRODUCTION

(+/-) 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) is a ring-substituted methamphetamine (METH) derivative and a common drug of abuse (Figure 1). MDMA elicits subjective effects in abusers including euphoria, increased empathy, and psychotropic alterations via its unique stimulation of neurotransmitter release (Gudelsky & Yamamoto, 2008). It has been well-established that MDMA binds the serotonin transporter (SERT) with high affinity, inhibiting and reversing its function, leading to the rapid release of high levels of serotonin (5-HT) into the synapse (Kuczenski, Segal, Cho, & Melega, 1995). MDMA has a similar, but lesser, effect on the dopamine (DAT) and norepinephrine transporters (Kuczenski et al., 1995), and has also been shown to promote the release of the neurotransmitter acetylcholine in a mechanism secondary to the release of 5-HT and dopamine (Nair & Gudelsky, 2006).

A well-documented, long-term effect of MDMA in the brain is a significant reduction in biochemical markers for 5-HT axon terminals, as evidenced by reductions in the tissue concentration of 5-HT and its metabolites, SERT protein levels, and immunoreactivity of the 5-HT nerve terminals across multiple brain regions, including the striatum, cortex, and hippocampus (Yamamoto & Raudensky, 2008). These findings have been reproduced in a number of species, including rats, nonhuman primates, and human abusers of the drug (Yamamoto & Raudensky, 2008), while in the mouse this toxicity is directed toward dopaminergic rather than serotonergic nerve terminals (Logan, Laverty, Sanderson, & Yee, 1988). This serotonergic toxicity has been found to persist for prolonged durations; although there is evidence of recovery in the cortex of rats at 24 weeks following MDMA exposure, depletion is still evident in the hippocampus (Adori et al., 2010). In a study of nonhuman primate MDMA exposure, Ricaurte, Martello, Katz, and Martello (1992) found that most brain regions examined exhibited depletions in 5-HT and its metabolite, 5-hydroxyindoleacetic acid, 18 months following drug administration. Abnormally high 5-HT levels indicated a hyperinnervation of the few brain regions that showed recovery at 18 months, suggesting that any recovery that may occur following MDMA may be functionally compromised.

Evidence for the persistence of MDMA 5-HT toxicity in humans is less consistent, as some studies document recovery in SERT levels shortly following abstinence (Erritzoe et al., 2011; Selvaraj et al., 2009; Thomasius et al., 2006), while other studies report elevated expression of 5-$HT_{2A}$ receptors in the cortex persisting up to two years following the last exposure to MDMA (Benningfield & Cowan, 2013; Di Iorio et al., 2012). It was proposed in these studies that the elevated expression levels of postsynaptic 5-$HT_{2A}$ receptors reflect chronically reduced 5-HT release. Such long-term decreases in serotonergic activity are thought to underlie persistent mood alterations observed in former users of MDMA, including depression and anxiety (Thomasius et al., 2006).




Methamphetamine 3,4-Methylenedioxymethamphetamine (MDMA)

**FIGURE 1 The comparative structures of methamphetamine and MDMA.** Methamphetamine (left) has a methyl group bound to the amine of its precursor, amphetamine. In addition to the methyl group, MDMA (right) contains a methylenedioxy side chain at the third and fourth carbons of the benzene ring. These structural differences impart a unique binding and toxicity profile to each compound.

## MECHANISMS OF MDMA 5-HT TOXICITY

The mechanism underlying MDMA-induced 5-HT toxicity is thought to primarily involve oxidative stress. MDMA is known to generate reactive oxygen and nitrogen species in the brain regions in which toxicity has been observed (Yamamoto & Raudensky, 2008). These oxidative species are thought to result, in part, from the abnormal uptake of dopamine into 5-HT terminals due to the alteration in SERT function induced by MDMA, as both pharmacological depletion of dopamine and blockade of its release was shown by Stone, Johnson, Hanson, and Gibb (1988) to attenuate the depletion of 5-HT evoked by MDMA. It was proposed in the same study that dopamine undergoes autooxidation to generate reactive oxygen and quinone species, which promote protein and lipid peroxidation in nerve terminals. In support of this hypothesis, multiple studies have demonstrated that the administration of antioxidants, such as ascorbic acid and cysteine, provide neuroprotection against the depletion of 5-HT evoked by MDMA administration in rodents (Gudelsky, 1996; Shankaran, Yamamoto, & Gudelsky, 2001). Alternatively, thioether metabolites of MDMA itself may function as reactive neurotoxicants (Monks, Jones, Bai, & Lau, 2004). For a more detailed mechanistic discussion of oxidative damage and MDMA serotonergic toxicity refer to the review by Yamamoto and Raudensky (2008).

## MDMA AND GAMMA-AMINOBUTYRIC ACID TOXICITY

The reduction in markers of 5-HT have been interpreted as a loss of fine 5-HT axonal projections (axotomy), while the cell bodies of 5-HT neurons themselves have been thought to be spared (Gudelsky & Yamamoto, 2003). However, studies support the view that MDMA produces toxicity toward cell bodies of neurons in selective brain regions. Capela et al. (2013; 2007) reported cell death in rat cortical and hippocampal neuron cultures following MDMA exposure. In addition, Schmued (2003) demonstrated cell death in the rat forebrain utilizing Fluoro-Jade B staining, while Soleimani Asl et al. (2012) reported increased *bax* and decreased *bcl-2* gene expression patterns in the rat hippocampus following MDMA exposure in vivo, indicating an increase in neuronal apoptotic signal. Additionally, MDMA-mediated increases in the apoptotic markers caspase-3 and calpain-1 have been demonstrated in rat cortical and hippocampal tissue (Wang et al., 2009; Warren et al., 2007). Further work has shown other indicators of apoptotic activation in the rat forebrain subsequent to MDMA exposure, including increased TUNEL staining and cytochrome C release (Wang et al., 2009).

Despite the aforementioned evidence indicative of MDMA-induced cell loss, there are relatively few studies that have addressed the specific neuronal populations impacted by repeated exposure to MDMA. Data are supportive of the view that MDMA-induced neurotoxicity extends beyond 5-HT axon terminals to gamma-aminobutyric acid (GABA) neurons in the hippocampus. Perrine et al. (2010) reported that the concentration of the inhibitory neurotransmitter GABA was decreased in the rat hippocampus following MDMA administration. In addition, a binge regimen of MDMA has been shown to significantly reduce the number of parvalbumin-positive GABA neurons in the dentate gyrus of the hippocampus (see Figure 2) (Anneken, Cunningham, Collins, Yamamoto, & Gudelsky, 2013). Moreover, repeated MDMA treatment has been shown to reduce glutamate decarboxylase 67 (GAD67) mRNA within the hippocampus (Kyser et al., 2011), as well as the number of GAD67-positive neurons within this brain region (Anneken, Huff, et al., 2013). Evidence of MDMA-induced deficits in hippocampal GABA neurons is not limited to rodent studies. Human abusers of MDMA also exhibit an impairment in GABA interneuron signaling in the hippocampus (Jacobsen, Mencl, Pugh, Skudlarski, & Krystal, 2004). Inasmuch as the hippocampus is a critical brain region for cognitive function, an understanding of MDMA-induced damage to neuronal populations within this structure may provide an insight into the neurochemical abnormalities underlying cognitive impairment in human abusers of MDMA. These implications will be discussed later in this chapter.

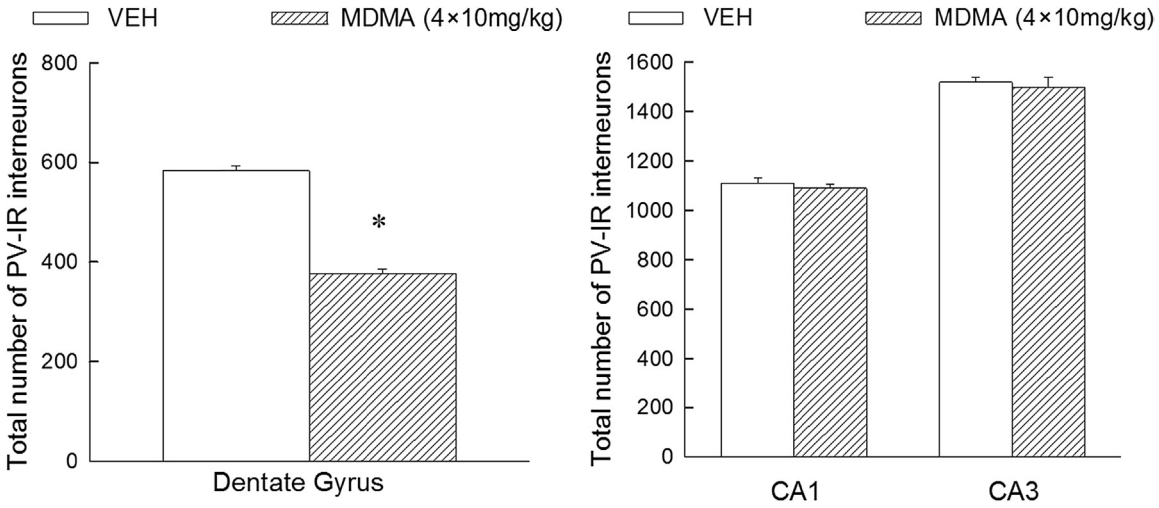



**FIGURE 2** **MDMA decreases the number of parvalbumin-positive GABA neurons in the rat dentate gyrus.** Administration of a binge regimen of MDMA (4×10 mg/kg given every 2 h) evoked a significant decrease in the number of parvalbumin-positive GABA neurons in the dentate gyrus of adult male rats. This effect was not observed in either the CA1 or CA3 regions in the same animals. N = 6 animals/group, +/− SEM.

Although the mechanisms underlying the MDMA-induced damage to hippocampal GABA neurons remain to be conclusively addressed, evidence suggests the involvement of 5-$HT_2$ receptors and neuroinflammatory processes. Anneken, Huff, et al. (2013) reported that the 5-$HT_{2A}$ antagonist MDL100907 prevented the MDMA-induced reduction in parvalbumin-positive GABA neurons in the hippocampus. In addition, the MDMA-induced reduction of hippocampal GABA neurons was shown by these authors to be diminished in rats treated with the nonselective cyclooxygenase (COX) inhibitor ketoprofen; however, the MDMA-induced depletion of hippocampal 5-HT was not prevented by ketoprofen administration. Hence, neuroinflammatory processes, particularly those involving COX, appear to contribute to MDMA-induced GABAergic, but not serotonergic, neurotoxicity.

Previous studies also have provided evidence to support a role of neuroinflammation in neurotoxicity elicited by amphetamine analogues. Kuhn, Francescutti-Verbeem, and Thomas (2006) have described a role for dopamine quinones in dopaminergic and serotonergic toxicity wherein microglia are activated by these toxicants to promote the formation of inflammatory cytokines. Indeed, treatment with METH or MDMA results in an activation of microglia (Escubedo et al., 1998; Kuhn et al., 2006; Orio et al., 2004). Activated microglia are generally believed to promote the secretion of a variety of reactive species, including cytokines, prostaglandins, and nitric oxide, that are potential neurotoxicants (Hanisch, 2002). Additional evidence supporting a role for microglia in amphetamine toxicities is the finding that minocycline, an inhibitor of microglial activation, prevents MDMA-induced 5-HT neurotoxicity (Orio et al., 2010; Zhang, Shirayama, Shimizu, Iyo, & Hashimoto, 2006).

The neuroinflammatory processes that contribute to amphetamine neurotoxicity may include an increased generation of cytokines. METH and MDMA increase the expression of cytokines such as IL-6, TNF-α, and IL-1β (Goncalves et al., 2008; Orio et al., 2010). Inhibition of MDMA-induced NFKβ activity and subsequent IL-1β formation reduces MDMA-induced 5-HT neurotoxicity (Orio et al., 2010).

Microglia activation also may be linked to an increased activity of COX, which may generate mediators of neurotoxicity. COX-2 activity was described as an obligatory factor in METH-induced dopamine neurotoxicity (Thomas & Kuhn, 2005). METH-induced dopamine toxicity is greatly diminished by ketoprofen, a nonselective inhibitor of COX, as well as in COX-2 knockout mice (Asanuma, Tsuji, Miyazaki, Miyoshi, & Ogawa, 2003; Thomas & Kuhn, 2005).

One mechanism whereby cytokines or COX-derived prostanoids may contribute to MDMA-induced neurotoxicity is through an enhancement of glutamate-mediated neurotoxicity. Zou and Crews (2005) demonstrated that TNF-α suppressed glutamate uptake, thereby promoting glutamate neurotoxicity. In addition, prostaglandins have been shown under in vitro and in vivo conditions to increase extracellular concentrations of glutamate (Anneken, Cunningham, et al., 2013; Bezzi et al., 1998).

## MDMA AND HIPPOCAMPAL GLUTAMATE

It is well documented that MDMA produces a rapid and relatively short-lived (e.g., 2–3 h) increase in the extracellular concentrations of dopamine and 5-HT in multiple brain regions such as striatum, prefrontal cortex, hippocampus, and nucleus accumbens. Although the release of dopamine and 5-HT evoked by MDMA is thought to involve reversal of the dopamine and 5-HT transporters, respectively, activation of 5-$HT_{2A/C}$ or 5-$HT_{2B/C}$ receptors by 5-HT is also thought to contribute to the magnitude of the evoked dopamine release (see Gudelsky & Yamamoto, 2008). Furthermore, acute MDMA treatment results in a rapid increase in the extracellular concentration of acetylcholine in the striatum, prefrontal cortex, and hippocampus that is relatively longer in duration (e.g., approximately 4 h) compared with the effects on dopamine and 5-HT release (see Gudelsky & Yamamoto, 2008).

Studies have documented the effects of MDMA on the extracellular concentration of glutamate. Although MDMA was shown to be ineffective in altering glutamate release in the striatum (Nash & Yamamoto, 1992), findings illustrate a stimulatory effect of MDMA on hippocampal glutamate release. In contrast to the stimulatory effect of a single injection of MDMA on extracellular dopamine, acetylcholine, and 5-HT, a single injection of MDMA does not alter extracellular glutamate in the hippocampus. However, the repeated administration of MDMA at 2 h intervals (binge regimen) results in a delayed and sustained increase in the extracellular concentration of extracellular glutamate in the dorsal hippocampus (Anneken & Gudelsky, 2012). A similar treatment regimen of METH has also been shown to elevate hippocampal glutamate release (Tata & Yamamoto, 2008). In addition, the effect of MDMA on extracellular glutamate appears to be relatively specific for the hippocampus, inasmuch as binge regimen MDMA treatment does not alter glutamate release in the striatum or prefrontal cortex (Anneken & Gudelsky, 2012). Hence, the temporal pattern of increase in extracellular glutamate elicited by MDMA, as well as pattern of brain regions in which this increase occurs, differs considerably from that for the elevation of dopamine, 5-HT, and acetylcholine (see Figure 3).

The release of hippocampal glutamate evoked by MDMA appears to be secondary to the increased release of 5-HT and the subsequent activation of 5-$HT_{2A/C}$ receptors. This contention is based on the observations that both fluoxetine and the 5-$HT_{2A/C}$ inhibitor ketanserin attenuate the stimulatory effect of MDMA on extracellular glutamate in the hippocampus (see Figure 4) (Anneken & Gudelsky, 2012). Although the neuronal circuitry involved in the hippocampal glutamate response to MDMA is unknown, it appears to involve a local effect of MDMA within the hippocampus. The exact nature of the involvement of 5-$HT_2$ receptors in mediating MDMA-induced glutamate release remains to be determined. However, it is known that 5-$HT_2$ receptor activation activates phospholipase A2 to promote the formation of arachidonic acid in astrocytes and that increased arachidonic acid concentrations can drive prostaglandin formation via COX (Mackowiak et al., 2002). Importantly, prostaglandins have been shown to stimulate glial glutamate release (Bezzi et al., 1998).

A role for COX in the mechanism underlying MDMA-stimulated glutamate release in the hippocampus has been proposed based on the finding that ketoprofen and the selective COX-2 inhibitor nimesulide prevent MDMA-induced hippocampal glutamate release (see Figure 4) (Anneken, Cunningham, et al., 2013). Thus, neuroinflammatory processes, as well as 5-$HT_2$ receptor activation, contribute to the mechanisms underlying the stimulatory effect of MDMA on hippocampal glutamate release.

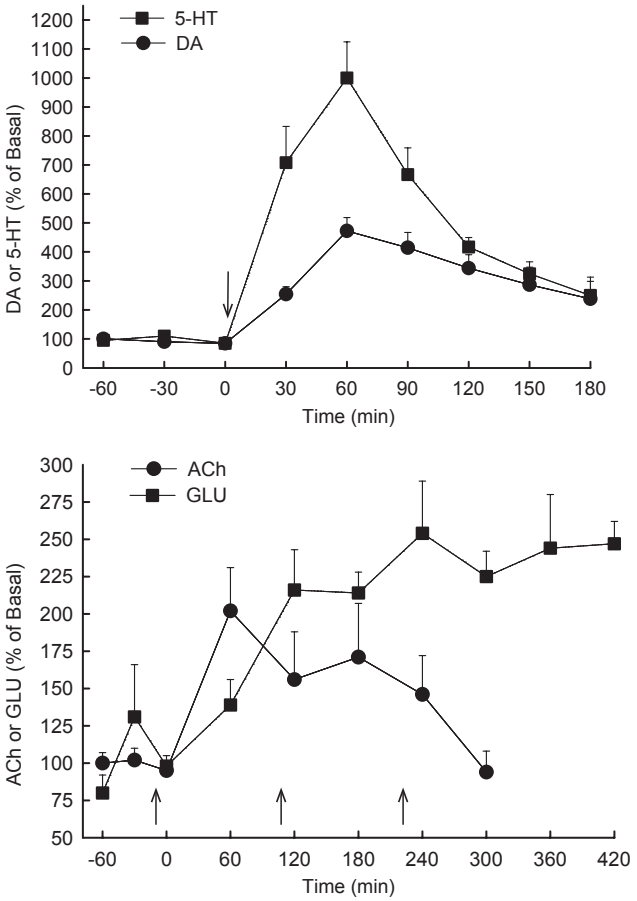



**FIGURE 3 Extracellular concentrations of neurotransmitters in the rat hippocampus following MDMA administration.** In the top panel, administration of a single dose of MDMA (10 mg/kg) to adult male rats elicits an increase in extracellular serotonin (5-HT) and dopamine (DA) in the hippocampus. In the bottom panel, administration of MDMA as a single dose (ACh data) or as a binge regimen (3 × 10 mg/kg given every 2 h; GLU data) elicits an increase in extracellular concentration of acetylcholine and glutamate, respectively. Data are presented as percent of a baseline value determined by averaging the three samples prior to MDMA administration. $N = 6–9$ animals/group, +/− SEM.

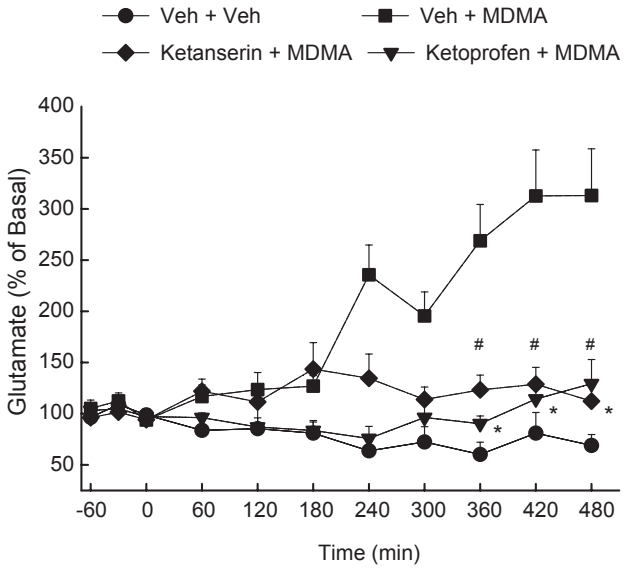



**FIGURE 4 Administration of ketoprofen or ketanserin attenuates the MDMA-evoked increase in extracellular hippocampal glutamate.** Adult male rats were given either the nonsteroidal anti-inflammatory drug ketoprofen (3 × 5 mg/kg, s.c.) or the 5-$HT_{2A/C}$ inhibitor ketanserin (2 × 3 mg/kg, intraperitoneally (i.p.)) or vehicle (Veh), along with MDMA (2 × 10 mg/kg, i.p., 2 h apart). Extracellular hippocampal glutamate, determined by microdialysis, is represented as a percentage of baseline levels on the graph, +/− SEM. #, * indicates a significant ($p < 0.05$) difference from vehicle + MDMA animals.

Although a causal relationship between MDMA-induced glutamate release and hippocampal GABA cell damage has not been established, several findings are supportive of this hypothesis. First, hippocampal parvalbumin-positive GABA neurons express group I metabotropic receptors (Kerner, Standaert, Penney, Young, & Landwehrmeyer, 1997), as well as alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors enriched in the GluR3 receptor subunit (Moga et al., 2003). These receptor subtypes are known to render neurons vulnerable to glutamate excitotoxicity. Secondly, blockade of 5-$HT_2$ receptors diminishes MDMA-stimulated glutamate release and MDMA-induced reductions in parvalbumin-positive GABA neurons. Finally, inhibition of COX also prevents both the MDMA-induced increase in glutamate release and the reduction in hippocampal GABA neurons (Anneken, Cunningham, et al., 2013; Anneken, Huff, et al., 2013). A hypothetical model that incorporates 5-$HT_2$ receptors, neuroinflammatory processes, and glutamate in MDMA-induced GABA neurotoxicity in the hippocampus is presented in Figure 5. MDMA-induced glutamate release may result from increased 5-HT release and activation of 5-$HT_2$ receptors, leading to activation of COX, a subsequent prostaglandin-mediated release of glial glutamate and excitotoxicity. An additional mechanism underlying MDMA-induced glutamate release and excitotoxicity of hippocampal GABA neurons may involve the induction of oxidative stress and nitric oxide production, reduction of endogenous antioxidants (e.g., glutathione), and subsequent activation of cystine–glutamate antiporter (system $X_c^-$) and increased efflux of glial glutamate.

## POTENTIAL CONSEQUENCES OF MDMA GABA TOXICITY

The functional implications of the reduction in GABA neurons following MDMA administration remain to be investigated. The hippocampus has been implicated in the cognitive dysfunction observed in both rats (Able, Gudelsky, Vorhees, & Williams, 2006; Piper & Meyer, 2004; Skelton et al., 2008) and human abusers of MDMA (den Hollander et al., 2012; Thomasius et al., 2006; Wagner, Becker, Koester, Gouzoulis-Mayfrank, & Daumann, 2013). A study by Jacobsen et al. (2004) provided preliminary evidence that the GABA interneurons that comprise the inhibitory circuits of the hippocampus are impaired in MDMA users, and as a result, it was proposed that this may underlie the cognitive dysfunction observed in the same human subjects. In support of that interpretation, den Hollander et al. (2012) reported a decrease

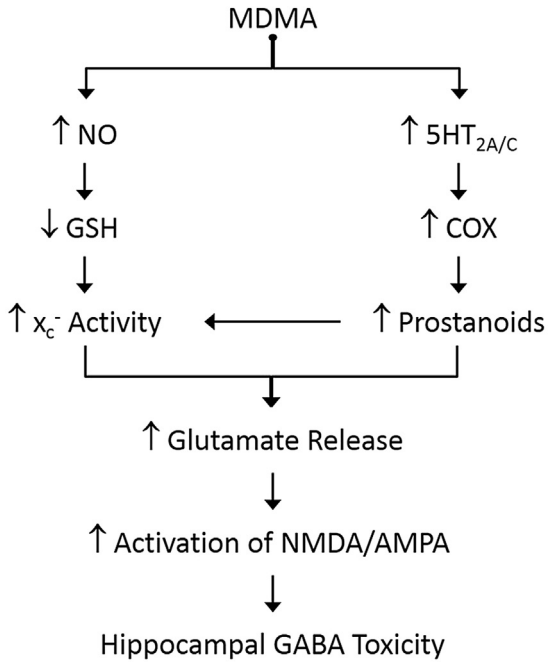



**FIGURE 5 Proposed integrated model of MDMA-induced glutamate release and GABAergic toxicity.** In this model, it is proposed that MDMA increases extracellular glutamate through two potential mechanisms and ultimately promotes excitotoxicity to hippocampal GABA neurons. First, stimulation of 5-$HT_{2A/C}$ receptors by the MDMA-induced release of 5-HT promotes increased COX activity leading to the production of prostaglandins and other proinflammatory signals which may directly stimulate astrocytic glutamate release or increase the activity of system $X_c^-$. Second, depletion of GSH following an MDMA-evoked increase in nitric oxide (NO) or hydroxyl radical formation could also drive $X_c^-$ function and lead to increased glutamate release. It is proposed that the elevated concentration of extracellular hippocampal glutamate activates ionotropic NMDA and AMPA receptors on GABA neurons in the dentate gyrus, ultimately leading to excitotoxic cell death.

in hippocampal volume in MDMA users compared with control, consistent with neuronal loss.

Alterations in the inhibitory input in the hippocampus could disrupt learning and memory. Cognitive impairments have been reported by Morellini et al. (2010) in transgenic mice with alterations to the balance of inhibitory and excitatory signaling due to an excess of GABA neurons. These mice display impairments in synaptic plasticity and long-term potentiation, particularly in the dentate gyrus. The increase in inhibitory signaling was found to alter animal performance in the Morris water maze, improving reversal learning. Based on this study, it is tempting to speculate that a reduction in dentate gyrus inhibitory signaling, such as would be expected following the MDMA-evoked loss of GABA neurons, would impair maze learning and potentially underlie verbal memory deficits in human users.

Another functional implication of the MDMA-evoked loss of hippocampal GABA neurons may be an increased vulnerability to seizures. Clinically, one of the most common acute complications resulting from MDMA use is the onset of seizures, but it has not been determined if the drug predisposes former users to chronic seizures (Brown, Dunne, Fatovich, Lee, & Lawn, 2011). Interestingly, mice exposed to a binge regimen of MDMA exhibit long-lasting alterations in electroencephalogram signals compared to saline-treated animals (Giorgi et al., 2005). Moreover, MDMA-treated animals are hypersensitive to the proconvulsant effects of the glutamate receptor agonist kainic acid. At both 2 and 8 weeks following exposure to MDMA, low, normally nonconvulsant doses of kainic acid were shown to elicit significant seizure activity in drug-treated mice compare to saline controls. This group of investigators also provided evidence of neuronal hyperexcitability in the hippocampus of MDMA-treated animals, consistent with an impairment in GABA signaling (Frenzilli et al., 2007). These findings support the hypothesis that the MDMA-evoked loss of GABA interneurons in the hippocampus results in a loss of inhibition and may predispose subjects to seizure activity.

## CONCLUSION

In conclusion, this review highlights findings that suggest a new target and mechanism for MDMA neurotoxicity. Beyond the well-documented induction of oxidative/bioenergetics stress produced by MDMA and the accompanying reduction in biochemical markers of 5-HT axon terminals (Table 1), studies suggest that MDMA damages neuronal cell bodies in selected brain regions. Indeed, repeated exposure to MDMA reduces markers for GABA neurons within the hippocampus. MDMA also produces a delayed, sustained, and selective increase in the extracellular concentration of glutamate in the hippocampus that may promote excitotoxic damage to vulnerable GABA neurons within this brain region (Table 1). These novel effects of MDMA within the hippocampus involve activation of 5-$HT_2$ receptors and neuroinflammatory processes (e.g., COX) that may act in concert to promote glial glutamate release. It remains to be determined whether glutamate-mediated excitotoxicity to GABA neurons within the hippocampus contributes to the cognitive impairment or altered seizure vulnerability that accompanies repeated exposure to MDMA.

## APPLICATIONS TO OTHER ADDICTIONS AND SUBSTANCE MISUSE

MDMA is a member of the amphetamine derivative class of drugs and, along with amphetamine and METH, illustrates the impact small changes in the structure of designer drugs can have on the effects these drugs have in the brain. Amphetamine is the most basic member of this class of drugs and has a very high binding affinity for the DAT, evoking a large release of dopamine through this transporter. Adding a methyl group to the amine of amphetamine produces METH, which has a roughly equivalent affinity for the DAT and SERT. As a result, METH releases a high amount of both 5-HT and dopamine and elicits unique subjective effects and toxicity on abusers. MDMA contains a methoxy group added to the benzene ring of METH, imparting a higher affinity for the SERT, a release of more 5-HT compared with dopamine, and consequently, its own unique subjective effects and toxicity. The near-endless chemical variations that can be made to the amphetamines present a particularly difficult challenge to drug enforcement policy, as the ongoing generation of new compounds, e.g., the newer "bath salts," can create functionally unique and potentially dangerous drugs of abuse.

**TABLE 1** Mechanisms of MDMA Neurotoxicity

| Mechanism | Evidence | Sources |
|---|---|---|
| Oxidative stress | MDMA increases oxidative species | Shankaran, Yamamoto, and Gudelsky (1999) and Sprague and Nichols (1995) |
| | 5-HT neuroprotection afforded by free radical spin-trapping agents and antioxidants | Colado and Green (1995), Gudelsky (1996), and Shankaran et al. (2001) |
| Bioenergetic stress | Altered glucose metabolism | Darvesh, Shankaran, and Gudelsky (2002) and Quate, McBean, Ritchie, Olverman, and Kelly (2004) |
| | Decreased ATP production | Darvesh and Gudelsky (2005) |
| | 5-HT neuroprotection afforded by energy supplementation | Darvesh and Gudelsky (2005) |
| Glutamate excitotoxicity | Increased extracellular glutamate in the hippocampus | Anneken and Gudelsky (2012) |
| | Reductions in excitotoxic-sensitive GABA neurons, GAD67 in the hippocampus | Anneken, Cunningham, et al. (2013) and Anneken, Huff, et al. (2013) |
| | GABA neuroprotection provided by drugs which also blunt MDMA glutamate release | Anneken, Cunningham, et al. (2013) and Anneken, Huff, et al. (2013) |
| Inflammation | Increased microglial activation | Orio et al. (2004) |
| | 5-HT and GABA neuroprotection afforded by anti-inflammatory drugs | Anneken, Cunningham, et al. (2013), Orio et al. (2010), and Zhang et al. (2006) |

Listed above are both the classic (bioenergetic and oxidative stress) and emerging (glutamate and inflammation) mechanisms thought to underlie MDMA neurotoxicity.

## DEFINITION OF TERMS

**Cyclooxygenase** It is an enzyme that produces proinflammatory mediators such as prostaglandins and thromboxanes. It is expressed as two major isoforms: COX-1, which is constitutively expressed; and COX-2, which is an inducible form.

**Dopamine** It is a neurotransmitter released by MDMA through its binding to the DAT. Dopamine promotes the elevated mood and hyperlocomotion experienced by MDMA users.

**Excitotoxicity** It is a neurotoxic process induced by the overstimulation of NMDA and AMPA glutamate receptors on postsynaptic neurons. The resulting influx of calcium ions both activates apoptotic signaling and promotes necrotic cell damage, ultimately leading to the death of the neuron.

**GABA** It is the primary inhibitory neurotransmitter in the brain. GABA neurons in the hippocampus are reduced following repeated MDMA exposure.

**Glutamate** It is the primary excitatory neurotransmitter in the brain. It is released by MDMA in a delayed, 5-HT- and inflammation-dependent manner.

**Hippocampus** It is a brain region critical for the formation and retention of memory. It is impacted by both serotonergic and GABAergic MDMA neurotoxicity.

**MDMA** It is a ring-substituted amphetamine derivative abused for its subjective effects on the user, including increased feelings of empathy and psychotropic hallucinations. These effects are mediated primarily by the release of 5-HT and dopamine. MDMA is toxic to 5-HT but not dopamine nerve terminals, and also elicits a toxic effect on GABA neurons.

**Methamphetamine** It is a commonly abused amphetamine derivative. This substance is highly addictive and promotes psychostimulant effects through the release of 5-HT and dopamine. METH also elicits serotonergic and dopaminergic neurotoxicity.

**Microdialysis** It is an experimental approach utilizing surgically implanted dialysis probes that allows the sampling of neurotransmitter levels in the awake, freely moving rat/mouse.

**Serotonin** It is a neurotransmitter released by MDMA through its binding to the serotonin reuptake transporter. 5-HT mediates the psychotropic effects felt by MDMA users. Neurotoxicity mediated by MDMA impacts 5-HT neurons throughout the forebrain.

**Serotonin reuptake transporter** SERT is a protein expressed on neurons which actively transports 5-HT from the synaptic cleft back into the cell for reuse or metabolism. It is the primary binding site for MDMA in the brain.

## KEY FACTS ABOUT MDMA ABUSE

- In 2013, it was estimated that between 5 and 13% of the population in the United States has tried MDMA in their lifetime. The great majority of those users are teenagers and young adults.
- MDMA is typically ingested orally as a pill or tablet and is most often taken during "rave" dance parties. Heavy users of MDMA will often stack several doses to prolong the effects of the drug.
- The most common acute side effect of MDMA intoxication is hyperthermia, which in extreme cases can be fatal.
- Negative long-term effects in MDMA users are thought to include depression, anxiety, and cognitive impairment.
- Despite its classification as a Schedule 1 drug, studies investigating its usefulness as an adjunct in psychotherapy have shown promising results in the treatment of anxiety disorders and posttraumatic stress disorder.

## SUMMARY POINTS

- MDMA is known to elicit an acute release of 5-HT, dopamine, acetylcholine, and norepinephrine across multiple brain regions, as well as glutamate specifically in the hippocampus.
- The release of hippocampal glutamate has been shown to be dependent upon serotonergic and neuroinflammatory mechanisms.
- MDMA elicits a persistent depletion of serotonergic markers across multiple brain regions, resulting primarily from oxidative stress.
- A novel reduction in GABA interneurons has been observed specifically in the hippocampus.
- Pharmacological blockade of 5-$HT_2$ receptors and inflammatory mediators prevents MDMA-evoked glutamate release and the reduction in GABA neurons, indicating that glutamate excitotoxicity may underlie the observed GABA toxicity.
- The observed loss of GABA interneurons could provide a mechanism for the cognitive impairments and increased seizure vulnerability observed following MDMA exposure.

## REFERENCES


Able, J. A., Gudelsky, G. A., Vorhees, C. V., & Williams, M. T. (2006). 3,4-Methylenedioxymethamphetamine in adult rats produces deficits in path integration and spatial reference memory. *Biological Psychiatry*, *59*(12), 1219–1226. http://dx.doi.org/10.1016/j.biopsych.2005.09.006.

Adori, C., Ando, R. D., Ferrington, L., Szekeres, M., Vas, S., Kelly, P. A., … Bagdy, G. (2010). Elevated BDNF protein level in cortex but not in hippocampus of MDMA-treated Dark Agouti rats: a potential link to the long-term recovery of serotonergic axons. *Neuroscience Letters*, *478*(2), 56–60. http://dx.doi.org/10.1016/j.neulet.2010.04.061.

Anneken, J. H., Cunningham, J. I., Collins, S. A., Yamamoto, B. K., & Gudelsky, G. A. (2013). MDMA increases glutamate release and reduces parvalbumin-positive GABAergic cells in the dorsal hippocampus of the rat: role of cyclooxygenase. *Journal of Neuroimmune Pharmacology*, *8*(1), 58–65. http://dx.doi.org/10.1007/s11481-012-9420-x.

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*(6), 1022–1027. http://dx.doi.org/10.1016/j.neuropharm.2012.07.026.

Anneken, J. H., Huff, C., Cunningham, J. I., Collins, S. A., Morano, R., Herman, J. P., … Gudelsky, G. A. (2013). MDMA reduces markers for GABAergic neurons in the hippocampus: role of 5–HT2 receptor- and cyclooxygenase-dependent glial glutamate release. Program number 157.20. In *Neuroscience meeting planner*. San Diego, CA: Society for Neuroscience. Online.

Asanuma, M., Tsuji, T., Miyazaki, I., Miyoshi, K., & Ogawa, N. (2003). Methamphetamine-induced neurotoxicity in mouse brain is attenuated by ketoprofen, a non-steroidal anti-inflammatory drug. *Neuroscience Letters*, *352*(1), 13–16.

Benningfield, M. M., & Cowan, R. L. (2013). Brain serotonin function in MDMA (ecstasy) users: evidence for persisting neurotoxicity. *Neuropsychopharmacology*, *38*(1), 253–255. http://dx.doi.org/10.1038/npp.2012.178.

Bezzi, P., Carmignoto, G., Pasti, L., Vesce, S., Rossi, D., Rizzini, B. L., … Volterra, A. (1998). Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. *Nature*, *391*(6664), 281–285. http://dx.doi.org/10.1038/34651.

Brown, J. W., Dunne, J. W., Fatovich, D. M., Lee, J., & Lawn, N. D. (2011). Amphetamine-associated seizures: clinical features and prognosis. *Epilepsia*, *52*(2), 401–404. http://dx.doi.org/10.1111/j.1528-1167.2010.02924.x.

Capela, J. P., da Costa Araujo, S., Costa, V. M., Ruscher, K., Fernandes, E., Bastos Mde, L., … Carvalho, F. (2013). The neurotoxicity of hallucinogenic amphetamines in primary cultures of hippocampal neurons. *Neurotoxicology*, *34*, 254–263. http://dx.doi.org/10.1016/j.neuro.2012.09.005.

Capela, J. P., Fernandes, E., Remiao, F., Bastos, M. L., Meisel, A., & Carvalho, F. (2007). Ecstasy induces apoptosis via 5-HT(2A)-receptor stimulation in cortical neurons. *Neurotoxicology*, *28*(4), 868–875. http://dx.doi.org/10.1016/j.neuro.2007.04.005.

Colado, M. I., & Green, A. R. (1995). The spin trap reagent alpha-phenyl-N-tert-butyl nitrone prevents 'ecstasy'-induced neurodegeneration of 5-hydroxytryptamine neurones. *European Journal of Pharmacology*, *280*(3), 343–346.

Darvesh, A. S., & Gudelsky, G. A. (2005). Evidence for a role of energy dysregulation in the MDMA-induced depletion of brain 5-HT. *Brain Research*, *1056*(2), 168–175. http://dx.doi.org/10.1016/j.brainres.2005.07.009.

Darvesh, A. S., Shankaran, M., & Gudelsky, G. A. (2002). 3,4-Methylenedioxymethamphetamine produces glycogenolysis and increases the extracellular concentration of glucose in the rat brain. *Journal of Pharmacology and Experimental Therapeutics*, *301*(1), 138–144.

den Hollander, B., Schouw, M., Groot, P., Huisman, H., Caan, M., Barkhof, F., & Reneman, L. (2012). Preliminary evidence of hippocampal damage in chronic users of ecstasy. *Journal of Neurology, Neurosurgery and Psychiatry*, *83*(1), 83–85. http://dx.doi.org/10.1136/jnnp.2010.228387.

Di Iorio, C. R., Watkins, T. J., Dietrich, M. S., Cao, A., Blackford, J. U., Rogers, B., … Cowan, R. L. (2012). Evidence for chronically altered serotonin function in the cerebral cortex of female 3,4-methylenedioxymethamphetamine polydrug users. *Archives of General Psychiatry*, *69*(4), 399–409. http://dx.doi.org/10.1001/archgenpsychiatry.2011.156.

Erritzoe, D., Frokjaer, V. G., Holst, K. K., Christoffersen, M., Johansen, S. S., Svarer, C., … Knudsen, G. M. (2011). In vivo imaging of cerebral serotonin transporter and serotonin(2A) receptor binding in 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") and hallucinogen users. *Archives of General Psychiatry*, *68*(6), 562–576. http://dx.doi.org/10.1001/archgenpsychiatry.2011.56.

Escubedo, E., Guitart, L., Sureda, F. X., Jimenez, A., Pubill, D., Pallas, M., … Camarasa, J. (1998). Microgliosis and down-regulation of adenosine transporter induced by methamphetamine in rats. *Brain Research*, *814*(1–2), 120–126.

Frenzilli, G., Ferrucci, M., Giorgi, F. S., Blandini, F., Nigro, M., Ruggieri, S., … 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. *Behavioural Pharmacology*, *18*(5–6), 471–481. http://dx.doi.org/10.1097/FBP.0b013e3282d518aa.

Giorgi, F. S., Pizzanelli, C., Ferrucci, M., Lazzeri, G., Faetti, M., Giusiani, M., … Fornai, F. (2005). Previous exposure to (+/-) 3,4-methylenedioxymethamphetamine produces long-lasting alteration in limbic brain excitability measured by electroencephalogram spectrum analysis, brain metabolism and seizure susceptibility. *Neuroscience*, *136*(1), 43–53. http://dx.doi.org/10.1016/j.neuroscience.2005.07.036.

Goncalves, J., Martins, T., Ferreira, R., Milhazes, N., Borges, F., Ribeiro, C. F., … Silva, A. P. (2008). Methamphetamine-induced early increase of IL-6 and TNF-alpha mRNA expression in the mouse brain. *Annals of the New York Academy of Sciences*, *1139*, 103–111. http://dx.doi.org/10.1196/annals.1432.043.

Gudelsky, G. A. (1996). Effect of ascorbate and cysteine on the 3,4-methylenedioxymethamphetamine-induced depletion of brain serotonin. *Journal of Neural Transmission*, *103*(12), 1397–1404.

Gudelsky, G. A., & Yamamoto, B. K. (2003). Neuropharmacology and neurotoxicity of 3,4-methylenedioxymethamphetamine. *Methods in Molecular Medicine*, *79*, 55–73.

Gudelsky, G. A., & Yamamoto, B. K. (2008). Actions of 3,4-methylenedioxymethamphetamine (MDMA) on cerebral dopaminergic, serotonergic and cholinergic neurons. *Pharmacology Biochemistry and Behavior*, *90*(2), 198–207. http://dx.doi.org/10.1016/j.pbb.2007.10.003.

Hanisch, U. K. (2002). Microglia as a source and target of cytokines. *Glia*, *40*(2), 140–155. http://dx.doi.org/10.1002/glia.10161.

Jacobsen, L. K., Mencl, W. E., Pugh, K. R., Skudlarski, P., & Krystal, J. H. (2004). Preliminary evidence of hippocampal dysfunction in adolescent MDMA ("ecstasy") users: possible relationship to neurotoxic effects. *Psychopharmacology (Berlin)*, *173*(3–4), 383–390. http://dx.doi.org/10.1007/s00213-003-1679-4.

Kerner, J. A., Standaert, D. G., Penney, J. B., Jr., Young, A. B., & Landwehrmeyer, G. B. (1997). Expression of group one metabotropic glutamate receptor subunit mRNAs in neurochemically identified neurons in the rat neostriatum, neocortex, and hippocampus. *Brain Research. Molecular Brain Research*, *48*(2), 259–269.

Kuczenski, R., Segal, D. S., Cho, A. K., & Melega, W. (1995). Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioral responses to the stereoisomers of amphetamine and methamphetamine. *Journal of Neuroscience*, *15*(2), 1308–1317.

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. *Annals of the New York Academy of Sciences*, *1074*, 31–41. http://dx.doi.org/10.1196/annals.1369.003.

Kyser, T. L., Hemmerle, A. M., Easley, K. E., Schmeltzer, S. N., Dickerson, J. W., Lundgren, K. H., … Seroogy, K. B. (2011). Acute effects of MDMA and methamphetamine treatment on expression of GAD mRNA in adult rat forebrain. In *Program number 472.09, Neuroscience meeting planner*. Washington, DC: Society for Neuroscience. Online.

Logan, B. J., Laverty, R., Sanderson, W. D., & Yee, Y. B. (1988). Differences between rats and mice in MDMA (methylenedioxymethylamphetamine) neurotoxicity. *European Journal of Pharmacology*, *152*(3), 227–234.

Mackowiak, M., Chocyk, A., Sanak, M., Czyrak, A., Fijal, K., & Wedzony, K. (2002). DOI, an agonist of 5-HT2A/2C serotonin receptor, alters the expression of cyclooxygenase-2 in the rat parietal cortex. *Journal of Physiology and Pharmacology*, *53*(3), 395–407.

Moga, D. E., Janssen, W. G., Vissavajjhala, P., Czelusniak, S. M., Moran, T. M., Hof, P. R., & Morrison, J. H. (2003). Glutamate receptor subunit 3 (GluR3) immunoreactivity delineates a subpopulation of parvalbumin-containing interneurons in the rat hippocampus. *Journal of Comparative Neurology*, *462*(1), 15–28. http://dx.doi.org/10.1002/cne.10710.

Monks, T. J., Jones, D. C., Bai, F., & Lau, S. S. (2004). The role of metabolism in 3,4-(+)-methylenedioxyamphetamine and 3,4-(+)-methylenedioxymethamphetamine (ecstasy) toxicity. *Therapeutic Drug Monitoring*, *26*(2), 132–136.

Morellini, F., Sivukhina, E., Stoenica, L., Oulianova, E., Bukalo, O., Jakovcevski, I., … Schachner, M. (2010). Improved reversal learning and working memory and enhanced reactivity to novelty in mice with enhanced GABAergic innervation in the dentate gyrus. *Cerebral Cortex*, *20*(11), 2712–2727. http://dx.doi.org/10.1093/cercor/bhq017.

Nair, S. G., & Gudelsky, G. A. (2006). Effect of a serotonin depleting regimen of 3,4-methylenedioxymethamphetamine (MDMA) on the subsequent stimulation of acetylcholine release in the rat prefrontal cortex. *Brain Research Bulletin*, *69*(4), 382–387. http://dx.doi.org/10.1016/j.brainresbull.2006.01.011.

Nash, J. F., & Yamamoto, B. K. (1992). Methamphetamine neurotoxicity and striatal glutamate release: comparison to 3,4-methylenedioxymethamphetamine. *Brain Research*, *581*(2), 237–243.

Orio, L., O'Shea, E., Sanchez, V., Pradillo, J. M., Escobedo, I., Camarero, J., … 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. *Journal of Neurochemistry*, *89*(6), 1445–1453. http://dx.doi.org/10.1111/j.1471-4159.2004.02443.x.

Orio, L., Llopis, N., Torres, E., Izco, M., O'Shea, E., & Colado, M. I. (2010). A study on the mechanisms by which minocycline protects against MDMA ('ecstasy')-induced neurotoxicity of 5-HT cortical neurons. *Neurotoxicity Research*, *18*(2), 187–199. http://dx.doi.org/10.1007/s12640-009-9120-3.

Perrine, S. A., Ghoddoussi, F., Michaels, M. S., Hyde, E. M., Kuhn, D. M., & Galloway, M. P. (2010). MDMA administration decreases serotonin but not N-acetylaspartate in the rat brain. *Neurotoxicology*, *31*(6), 654–661. http://dx.doi.org/10.1016/j.neuro.2010.08.005.

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. *Pharmacology Biochemistry and Behavior*, *79*(4), 723–731. http://dx.doi.org/10.1016/j.pbb.2004.10.001.

Quate, L., McBean, D. E., Ritchie, I. M., Olverman, H. J., & Kelly, P. A. (2004). Acute methylenedioxymethamphetamine administration: effects on local cerebral blood flow and glucose utilisation in the Dark Agouti rat. *Psychopharmacology (Berlin)*, *173*(3–4), 287–295. http://dx.doi.org/10.1007/s00213-004-1784-z.

Ricaurte, G. A., Martello, A. L., Katz, J. L., & Martello, M. B. (1992). Lasting effects of (+-)-3,4-methylenedioxymethamphetamine (MDMA) on central serotonergic neurons in nonhuman primates: neurochemical observations. *Journal of Pharmacology and Experimental Therapeutics*, *261*(2), 616–622.

Schmued, L. C. (2003). Demonstration and localization of neuronal degeneration in the rat forebrain following a single exposure to MDMA. *Brain Research*, *974*(1–2), 127–133.

Selvaraj, S., Hoshi, R., Bhagwagar, Z., Murthy, N. V., Hinz, R., Cowen, P., … Grasby, P. (2009). Brain serotonin transporter binding in former users of MDMA ('ecstasy'). *British Journal of Psychiatry*, *194*(4), 355–359. http://dx.doi.org/10.1192/bjp.bp.108.050344.

Shankaran, M., Yamamoto, B. K., & Gudelsky, G. A. (1999). Involvement of the serotonin transporter in the formation of hydroxyl radicals induced by 3,4-methylenedioxymethamphetamine. *European Journal of Pharmacology*, *385*(2–3), 103–110.

Shankaran, M., Yamamoto, B. K., & Gudelsky, G. A. (2001). Ascorbic acid prevents 3,4-methylenedioxymethamphetamine (MDMA)-induced hydroxyl radical formation and the behavioral and neurochemical consequences of the depletion of brain 5-HT. *Synapse*, *40*(1), 55–64. http://dx.doi.org/10.1002/1098-2396(200104)40:1<55::AID-SYN1026>3.0.CO;2-O.

Skelton, M. R., Able, J. A., Grace, C. E., Herring, N. R., Schaefer, T. L., Gudelsky, G. A., … Williams, M. T. (2008). (+/-)-3,4-Methylenedioxymethamphetamine treatment in adult rats impairs path integration learning: a comparison of single vs once per week treatment for 5 weeks. *Neuropharmacology*, *55*(7), 1121–1130. http://dx.doi.org/10.1016/j.neuropharm.2008.07.006.

Soleimani Asl, S., Farhadi, M. H., Moosavizadeh, K., Samadi Kuchak Saraei, A., Soleimani, M., Jamei, S. B., … Mehdizadeh, M. (2012). Evaluation of Bcl-2 family gene expression in hippocampus of 3, 4-methylenedioxymethamphetamine treated rats. *Cell Journal*, *13*(4), 275–280.

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. *Journal of Pharmacology and Experimental Therapeutics*, *273*(2), 667–673.

Stone, D. M., Johnson, M., Hanson, G. R., & Gibb, J. W. (1988). Role of endogenous dopamine in the central serotonergic deficits induced by 3,4-methylenedioxymethamphetamine. *Journal of Pharmacology and Experimental Therapeutics*, *247*(1), 79–87.

Tata, D. A., & Yamamoto, B. K. (2008). Chronic stress enhances methamphetamine-induced extracellular glutamate and excitotoxicity in the rat striatum. *Synapse*, *62*(5), 325–336. http://dx.doi.org/10.1002/syn.20497.

Thomas, D. M., & Kuhn, D. M. (2005). Cyclooxygenase-2 is an obligatory factor in methamphetamine-induced neurotoxicity. *Journal of Pharmacology and Experimental Therapeutics*, *313*(2), 870–876. http://dx.doi.org/10.1124/jpet.104.080242.

Thomasius, R., Zapletalova, P., Petersen, K., Buchert, R., Andresen, B., Wartberg, L., … Schmoldt, A. (2006). Mood, cognition and serotonin transporter availability in current and former ecstasy (MDMA) users: the longitudinal perspective. *Journal of Psychopharmacology*, *20*(2), 211–225. http://dx.doi.org/10.1177/0269881106059486.

Wagner, D., Becker, B., Koester, P., Gouzoulis-Mayfrank, E., & Daumann, J. (2013). A prospective study of learning, memory, and executive function in new MDMA users. *Addiction*, *108*(1), 136–145. http://dx.doi.org/10.1111/j.1360-0443.2012.03977.x.

Wang, X., Zhu, S. P., Kuang, W. H., Li, J., Sun, X., Huang, M. S., & Sun, X. L. (2009). Neuron apoptosis induced by 3,4-methylenedioxy methamphetamine and expression of apoptosis-related factors in rat brain. *Sichuan Da Xue Xue Bao Yi Xue Ban*, *40*(6), 1000–1002, 1037.

Warren, M. W., Larner, S. F., Kobeissy, F. H., Brezing, C. A., Jeung, J. A., Hayes, R. L., … Wang, K. K. (2007). Calpain and caspase proteolytic markers co-localize with rat cortical neurons after exposure to methamphetamine and MDMA. *Acta Neuropathologica*, *114*(3), 277–286. http://dx.doi.org/10.1007/s00401-007-0259-9.

Yamamoto, B. K., & Raudensky, J. (2008). The role of oxidative stress, metabolic compromise, and inflammation in neuronal injury produced by amphetamine-related drugs of abuse. *Journal of NeuroImmune Pharmacology*, *3*(4), 203–217. http://dx.doi.org/10.1007/s11481-008-9121-7.

Zhang, L., Shirayama, Y., Shimizu, E., Iyo, M., & Hashimoto, K. (2006). Protective effects of minocycline on 3,4-methylenedioxymethamphetamine-induced neurotoxicity in serotonergic and dopaminergic neurons of mouse brain. *European Journal of Pharmacology*, *544*(1–3), 1–9. http://dx.doi.org/10.1016/j.ejphar.2006.05.047.

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 Research*, *1034*(1–2), 11–24. http://dx.doi.org/10.1016/j.brainres.2004.11.014.