MDMA Neurotoxicity: Studies in Nonhuman animals
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Introduction and Overview

Numerous studies have examined nonhuman animals and tissue cultures for evidence of MDMA-induced neurotoxicity. These studies are important because they allow controlled investigation of toxic changes that may occur in humans. These studies can be divided into three areas of neurochemical investigation: (1) monoaminergic neurotoxicity; (2) non-monoaminergic neurotoxicity; and (3) *in vitro* decreases in neural cell viability. The possible damage identified in each of these areas cannot always be equated. Nonetheless, any study of functioning in intact MDMA-exposed animals implicitly investigates all types of neurotoxicity.

High or repeated-dose MDMA regimens can produce long-term changes in indices of monoaminergic and axonal functioning in animals. Increasing evidence indicates that these changes are at least partially the result of damage. The magnitude of these changes varies with dose, species, and route of administration. Rodent studies have shown that changes in the core temperature of animals can increase or decrease MDMA neurotoxicity, although this finding has not been confirmed in primates. While some recovery does occur, a study in squirrel monkeys suggests that there may be permanent changes in axonal distribution. Oxidative stress appears to play an important role in MDMA neurotoxicity, but the exact mechanisms are poorly understood. The sustained acute pharmacological effects of MDMA may exhaust neuronal energy sources and antioxidant defenses, leading to damage. Metabolites of MDMA are another possible source of oxidative stress. The risks of monoaminergic neurotoxicity in humans are controversial and are discussed in the next chapter.

Research has also uncovered MDMA-induced non-monoaminergic neurotoxicity in rats. Measures of neural cell injury indicate that MDMA, like methamphetamine, can damage non-monoaminergic cell bodies in the somatosensory cortex. Another area of research uses cultured cell lines and has suggested that sustained exposure to MDMA can decrease neural cell viability and trigger programmed cell death. These neural cell changes have only been detected after high MDMA exposures that are unlikely to occur in clinical settings.

Few behavioral correlates of neurotoxic MDMA exposure have been found in drug-free nonhuman animals, despite dramatic serotonergic changes, alterations in neurofunctioning, and changes in response to drugs. Changes in MDMA-exposed animals include thermoregulatory impairment, decreased locomotor activity, and neurocognitive impairment. Lasting thermoregulatory impairment has been demonstrated in MDMA-exposed animals by two research groups. Rats exposed to a neurotoxic MDMA regimen showed reductions in diurnal and nocturnal locomotor activity at 7 to 14 days after drug treatment. Two studies have suggested that neurotoxic MDMA exposure may cause neurocognitive impairment in rats. The first study used adult animals and the second study used newborn rats. In contrast, at least 9 other studies failed to find evidence of neurocognitive impairment in MDMA-exposed animals.

These studies indicate that neurotoxic MDMA exposures can cause behavioral changes. These changes have been difficult to detect and it is not known whether they are temporary or permanent.

This section will discuss: (1) the nature and interpretation of MDMA-induced serotonergic changes; (2) the possible mechanisms of these changes; (3) factors influencing the magnitude of these changes (such as dose, route of administration, species and animal strain, and environment); (4) the time course of these changes and recovery; (5) evidence of non-monoaminergic damage; (6) *in vitro* cell viability studies; and (7) the behavioral and functional correlates of MDMA-induced long-term changes in animals.

Definitions

Before discussing MDMA-induced changes and their interpretation, it is necessary to define a few terms. In this document, drug doses and dosing patterns that produce these long-term serotonergic changes will be referred to as "neurotoxic regimens." Neurotoxic regimens often consist of four to eight injections of MDMA given over the course of one to four days. However, a single injection of MDMA can also produce these changes. In this document, any changes noted at 7 or more days after drug administration will be considered "long-term." Enough studies have also examined the brains of animals at longer time periods (often at 2, 4, or 8 weeks) to establish that the MDMA-induced serotonergic changes at 7 days are primarily long-term in nature.

The reader will note that the term "neurotoxicity" has not been defined. There are, unfortunately, no universally accepted definitions of this term and most definitions are broad enough to encompass short-term alcohol-induced headaches as well as the permanent nerve cell damage and parkinsonism caused by the neurotoxic meperidine analogue N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). A useful approach to the question of whether MDMA is neurotoxic may be to describe the nature and mechanisms of the long-term changes it can cause. When this is done, it can be seen that the serotonergic changes induced by at least some neurotoxic MDMA regimens are accompanied by loss of axons and that the acute events that trigger these changes involve damage to the brain by free radicals. This suggests that MDMA neurotoxicity is a type of drug-induced damage, even though the consequences of this damage are elusive. It must be noted that some scientists disagree with this interpretation and argue that MDMA-induced serotonergic changes should not be considered neurotoxic. This matter will be discussed below.

MDMA Can Induce Long-term Serotonergic Changes

At some, but not all, active doses, MDMA produces long-lasting changes to the serotonergic system. These long-term changes include decreases in brain concentrations of the neurotransmitter serotonin (5HT) and its metabolite 5-hydroxyindoleacetic acid (5HIAA). Tryptophan hydroxylase (TPH), the rate-limiting enzyme within the serotonergic neuron that begins the synthesis of 5HT, is decreased. There are also decreases in the density of the

serotonin reuptake transporter (SERT). SERT is a protein on the membrane of serotonergic neurons that functions to "recycle" released 5HT by transferring it back into the serotonergic neuron. Most studies suggest that MDMA primarily causes long-term changes in serotonergic neurons that have their cell bodies in an area of the brainstem called the dorsal raphe nucleus.

Long-lasting decreases in these serotonergic markers suggest that either (a) some type of "down-regulation" has occurred and the nerve cell is making and maintaining fewer of the markers or (b) that there are fewer serotonergic nerve terminals and axons in the region being measured. This issue has been important to the question of whether MDMA is truly neurotoxic. Down-regulation suggests an active adaptation to drug effects, while axonal loss suggests damage may have occurred. Deciding between these possibilities can be difficult. SERT density can be regulated in response to drugs, although this has been difficult to consistently demonstrate experimentally (Le Poul et al. 2000; Ramamoorthy et al. 1998). Similarly, 5HT levels can be influenced by diet and other factors. Because MDMA has been shown to rapidly inactivate the enzyme TPH, decreased 5HT levels would be expected until TPH activity returns to normal. Thus, decreased 5HT synthesis and subsequent SERT down-regulation initially appear to be a plausible explanation for MDMA-induced serotonergic changes. Ultimately, however, it is clear that MDMA can cause axonal loss. To demonstrate this, it is necessary to examine the structure of serotonergic axons and terminals in MDMA-exposed animals.

Serotonergic Changes are Accompanied by Structural Changes to Axons

An important approach to understanding MDMA-induced serotonergic changes involves staining brain slices from MDMA-exposed animals. Most commonly, immunocytochemistry techniques are used to stain 5HT, allowing serotonergic axons and terminals to be seen. When this is done, irregular swelling and what appears to be fragmentation of fine serotonergic axons is visible shortly after a neurotoxic regimen of MDMA or MDA (Kalia et al. 2000; O'Hearn et al. 1988; Scallet et al. 1988). Later immunocytochemistry measurements, at 2 or 4 weeks after neurotoxic MDMA regimens, also show a persistent decrease in stained axons (O'Hearn et al. 1988; Scallet et al. 1988; Slikker et al. 1988; Wilson et al. 1989). The initial swelling suggests some type of axonal damage, while the later decrease in stained axons suggests a loss of axons. However, some have argued that immunocytochemistry cannot reliably distinguish between true changes in 5HT-containing axons and changes in 5HT that are unaccompanied by axonal change. Because of this limitation, it is necessary to confirm the apparent loss of axons using techniques that do not rely on serotonergic markers.

Transport of materials within axons is crucial for maintaining cell structure and function. Lasting reductions in axonal transport suggest a drastic impairment of axonal functioning and, more likely, loss of axons. One can assess axonal transport by measuring transport of compounds between brain regions that serotonergic axons should connect. For example, if injected into the cortex, the fluorescent dye Fluoro-Gold should be transported along serotonergic axons into cell bodies in the brainstem. Axonal transport studies have been carried out after neurotoxic MDMA (Callahan et al. 2001; Ricaurte et al. 2000) and

parachloroamphetamine (Fritschy et al. 1988; Haring et al. 1992) regimens. Their results suggest that a loss of axons occurs after at least some neurotoxic regimens of MDMA and related drugs.

Another method of assessing loss of nerve terminals involves measuring the vesicular monoamine transporter type II (VMAT2). This is a protein on the storage vesicles inside serotonergic (and other monoaminergic) nerve terminals. Because the amount of VMAT2 does not appear to be adjusted in response to drug exposure (Vander Borght et al. 1995), it is sometimes used as an indirect measure of nerve terminals in research on neurodegenerative disorders such as Parkinson's disease. In the case of neurotoxic MDMA exposure, decreased VMAT2 would suggest that nerve terminals and axons have been lost. In fact, neurotoxic regimens of MDMA (Ricaurte et al. 2000) or methamphetamine (Frey et al. 1997) can decrease VMAT2. Therefore, at least some neurotoxic regimens of MDMA are associated with structural changes to cells. Examining the dorsal raphe nucleus leads to the conclusion that cell bodies of these affected axons do not die despite axon loss (Fischer et al. 1995; Hatzidimitriou et al. 1999; O'Hearn et al. 1988).

The data presented so far consistently indicate that MDMA can cause serotonergic axons to degenerate and that this explains at least some of the MDMA-induced decrease in serotonergic markers. Further evidence of axonal degeneration comes from studies in which recovery from MDMA neurotoxicity is associated with apparent sprouting and regrowth of axons (discussed in more detail below). At this point the reader may be wondering why MDMA neurotoxicity has been controversial. There are probably three reasons. First, research on axonal transport and VMAT2 has only recently been carried out with MDMA. Second, MDMA neurotoxicity initially seemed to be without any behavioral correlates. Third, techniques that normally detect neural cell damage yield ambiguous results after MDMA regimens. This point is discussed below.

Non-serotonergic Indicators of Cell Damage are Inconsistently Affected by MDMA

In general, neural cell damage can be detected using silver staining and/or by measuring expression of glilal fibrillary acidic protein (GFAP) (O'Callaghan et al. 1995). These techniques seem to detect MDMA-induced alterations at higher doses than those needed to affect serotonergic indices (Commins et al. 1987b; O'Callaghan and Miller 1993). In one study, very high neurotoxic MDMA exposures resulted in increased GFAP but produced less 5HT depletion than lower MDMA exposures (O'Callaghan and Miller 1993). Furthermore, the MDMA-induced cell damage detected by silver staining appears to occur in nonserotonergic cells (Commins et al. 1987b; Jensen et al. 1993) as well as in what are likely serotonergic axons (Scallet et al. 1988). These inconsistencies are difficult to interpret. Some have interpreted them as evidence that MDMA-induced serotonergic changes are the result of down-regulation of the serotonergic system rather than damage (e.g., O'Callaghan and Miller in press). Others have argued that the techniques for measuring cell damage are simply insensitive to selective serotonergic damage (Axt et al. 1994; Bendotti et al. 1994; Wilson and Molliver 1994). MDMA-induced damage to non-serotonergic cells is discussed in more detail in a subsequent section.

Because studies of axonal transport and VMAT2 changes have provided strong evidence of MDMA-induced axonal degeneration, it appears that serotonergic down-regulation can no longer fully explain the long-term effects of MDMA. Structural changes to serotonergic axons must also be explained. Although we are not aware that this hypothesis has been advanced, one could argue that loss of axons represents a non-neurotoxic form of neuroplasticity. In fact, non-neurotoxic (though not necessarily beneficial) morphological changes can occur in the CNS as the result of alterations in serotonin levels (reviewed in Azmitia 1999). However, as we better understand the mechanisms of these MDMA-induced serotonergic changes, it appears more likely that these changes are, in fact, the result of damage, specifically damage involving oxidative stress.

The Role of Oxidative Stress in MDMA neurotoxicity

Neurotoxic regimens of MDMA increase oxidative stress in the brain. In this document, the term "oxidative stress" will be used to refer to both the increase in free radicals and other reactive chemical species and the burden these species place on cellular functioning. Free radicals are highly reactive chemical species that contain one or more unpaired electrons and exist separately. Free radicals can damage neural macromolecules through reduction and oxidation reactions, altering the ability of these molecules to carry out their normal cellular function.

MDMA-induced oxidative stress has been measured in two ways. First, researchers have examined the brains of MDMA-treated animals for substances that react with thiobarbituric acid (Colado et al. 1997a; Jayanthi et al. 1999; Sprague and Nichols 1995b). Increases in these substances suggest that neural lipids have been oxidized. Second, researchers have perfused the brains of live animals with either salicylate or d-phenylalanine. These substances react with hydroxyl radicals to form 2,3-dihydroxybenzoic acid and d-tyrosine, respectively. By measuring formation of these compounds, researchers have demonstrated that neurotoxic MDMA regimens increase the amount of extracellular hydroxyl radicals of the striatum (Shankaran et al. 2001; Shankaran et al. 1999a; b) and hippocampus (Colado et al. 1999b; Colado et al. 1997b).

There is strong evidence that this oxidative stress is involved in the mechanisms of MDMA neurotoxicity. The antioxidants, ascorbate and cysteine, each reduce MDMA neurotoxicity in rats without altering striatal levels of MDMA or MDMA-stimulated dopamine release (Gudelsky 1996; Schmidt and Kehne 1990; Shankaran et al. 2001; Shankaran et al. 1999a; b). Ascorbate also decreases the acute MDMA-induced oxidation of endogenous vitamin E in the striatum and hippocampus (Shankaran et al. 2001). The free radical scavenger N-tert-butyl-alpha-phenylnitrone decreases both MDMA-induced hydroxyl formation and MDMA neurotoxicity in rats, although this may be partially due to an attenuation of MDMA-induced hyperthermia (Che et al. 1995; Colado et al. 1998; Colado and Green 1995; Yeh 1999). Pretreatment with the antioxidant alpha-lipoic acid blocks both MDMA-induced serotonergic neurotoxicity and increased GFAP expression in the rat hippocampus without altering MDMA-induced hyperthermia (Aguirre et al. 1999). Mice that have been genetically altered to have large amounts of the human antioxidant enzyme, copper/zinc superoxide dismutase, are protected from MDMA-induced dopamine depletions, probably because of the increased trapping of superoxide

radicals (Cadet et al. 1994; Cadet et al. 1995; Jayanthi et al. 1999). At the same time, these genetically modified mice are protected from the acute inactivation of antioxidant enzymes and increases in neural lipid peroxidation seen in normal mice after a neurotoxic MDMA regimen (Cadet et al. 1994; Cadet et al. 1995; Jayanthi et al. 1999).

Early evidence that MDMA caused significant oxidative stress (Stone et al. 1989a) showed that TPH that had been inactivated in rats at 3 hrs after high dose MDMA could be reactivated *in vitro* using sulfhydryl-reducing conditions. This demonstrated that the acute inactivation of TPH by MDMA was due to intracellular oxidative stress. Intracellular oxidative stress appears to be an effect of MDMA that requires sustained brain concentrations of MDMA (or a centrally formed metabolite). While a single injection of MDMA into the brain (intracerebroventricularly) had no effect on TPH activity, slow infusion of 1 mg/kg MDMA into the brain over 1 hr produced enough oxidative stress to acutely reduce TPH activity (Schmidt and Taylor 1988). The acute decrease in TPH activity is an early effect of MDMA and can be measured at post 15 min (Stone et al. 1989b). TPH inactivation can also be produced by non-neurotoxic MDMA doses (Schmidt and Taylor 1988; Stone et al. 1989a; Stone et al. 1989b). It therefore appears that MDMA rapidly induces oxidative stress but only produces neurotoxicity when endogenous free radical scavenging systems are overwhelmed.

In summary, MDMA neurotoxicity involves an initial period of oxidative damage, with increases in free radicals and damage to neural lipids occurring. It appears difficult to argue that dramatic MDMA-induced increases in free radicals and resulting oxidation of neural lipids and proteins are not damage. This damage seems to be part of the sequence of events producing serotonergic neurotoxicity since treatments that decrease MDMA-induced oxidative stress also decrease the long-term serotonergic changes (e.g., Aguirre et al. 1999). While MDMA can cause loss of axons, some simultaneous serotonergic down-regulation cannot be ruled out. Research on methamphetamine-induced dopaminergic neurotoxicity has led some to conclude that long-term dopaminergic changes can occur without significant axonal loss (Harvey et al. 2000; Wilson et al. 1996). Whether this is also the case with MDMA is unknown. For now, it seems reasonable to consider long-term serotonergic alterations after MDMA exposure as indicating that some degree of damage has occurred, while remembering that one is also measuring the response of the serotonergic system to acute drug effects and loss of axons.

Proposed Sources of Oxidative Stress

Several possible sources of neurotoxic oxidative stress have been proposed. First, the sustained pharmacological effects of MDMA may deplete neuronal energy sources and/or impair energy metabolism within the neuron (Huether et al. 1997). Second, both MDMA and dopamine can be metabolized to highly reactive quinone-like molecules. Quinones are molecules with two carbonyl groups either adjacent or separated by two carbons on an unsaturated six-membered ring. They are often very reactive and can form free radicals, potentially reacting with and damaging neural macromolecules. There is not yet conclusive evidence to implicate any of these possible causes and some (perhaps regionally specific) combination of mechanisms is possible. The possible roles of energy exhaustion or impairment, MDMA metabolites, and dopamine

metabolites are discussed below. It has also been proposed that 5HT metabolites, increased intracellular Ca2+, nitric oxide, or glutamate may contribute to MDMA neurotoxicity. However, current evidence provides little support for these theories and their discussion will be brief.

Energy Exhaustion or Impairment as a Source of Oxidative Stress

Energy exhaustion or impairment may cause MDMA neurotoxicity. The normal activities of the neuron cause a certain degree of oxidative stress. A sustained increase in neuronal activity would therefore be expected to increase oxidative stress. More importantly, increased neuronal activity is accompanied by increased energy consumption that could eventually lead to a depletion of neuronal energy sources. This can impair the energy-requiring mechanisms that maintain and repair neurons. Furthermore, the most important source of cellular energy, mitochondria, can be impaired by oxidative stress (Crompton et al. 1999). Mitochondria produce adenosine triphosphate (ATP), the source of energy for most cellular processes. Insufficient ATP will lead to cell damage or death.

Whether energy exhaustion or impairment actually plays a role in MDMA neurotoxicity is not yet clear. MDMA has been shown to increase neuronal energy consumption. In rats, doses of 5 to 30 mg/kg intraperitonal MDMA were found to acutely (post 50 min) increase cerebral glucose utilization in 12 of 60 examined regions, while decreasing utilization in 8 regions (Wilkerson and London 1989). The measurement time used in this study was likely too early to detect possible neurotoxicity-related energy depletion. MDMA also increases glycogen phosphorylase activity *in vitro* (Poblete and Azmitia 1995), which suggests that MDMA could decrease glial stores of glycogen, an important source of energy in the brain.

MDMA-induced alterations in mitochondria functioning have been reported (Burrows et al. 2000), but it is not yet clear these alterations are sufficient to impair mitochondria and damage cells. Burrows, Gudelsky, and Yamamoto (2000) reported that a neurotoxic regimen of MDMA transiently inhibited by 10 to 20% the activity of cytochrome oxidase, one of the protein complexes catalyzing oxidative phosphorylation. It is not clear if this degree of inhibition significantly impairs mitochondrial functioning. In another study, brain ATP levels were unchanged at 1 to 3 hours after a neurotoxic dose of MDMA to rats (Hervias et al. 2000). This shows that the ability of mitochondria to produce ATP is not impaired at these times, although later times were not examined. In the same report, nicotinamide increased MDMA neurotoxicity. Nicotinamide is the precursor molecule for the electron carrier NAD. It should therefore have enhanced ATP production and reduced neurotoxicity if mitochondrial impairment is truly involved. However, the authors suggest that nicotinamide may also alter MDMA metabolism, increasing formation of neurotoxic metabolites. At this point, evidence that MDMA neurotoxicity involves mitochondrial impairment must be considered inconclusive.

MDMA Metabolites as a Source of Oxidative Stress

MDMA metabolites may also play a role in MDMA neurotoxicity. However, it is difficult to investigate this possible role. Hypothetically, a given metabolite may only be toxic in the

Table 4.1. Studies of the Neurotoxicity of Putative MDMA Metabolites

PUTATIVE METABOLITE	DOSE	ROUTE	5HT	5HIAA	TPH	DA	TH	DOPAC/HVA	OTHER CHANGES	REFERENCE
2,5-bis(glutathion-S-yl)-alphamethyldopamine	150 nmol X 4, every 12 hrs	intracortical	Decreased in STR and CORT. No significant change in HIP, MID/DI/TEL and PONS.	No significant change in CORT, STR, HIP, MID/DI/TEL and PONS	NA	No significant changes in CORT, STR, or HIP.	NA	NA	No significant change in NE in STR or HIP.	Bai et al., 1999
2,5-bis(glutathion-S-yl)-alphamethyldopamine	300 nmol X 4, every 12 hrs	intracortical	Decreased in CORT and STR. No significant change in HIP, midbrain/diencephalon/telencephalon, and PONS.	No significant change in CORT, STR, HIP, MID/DI/TEL and PONS.	NA	No significant changes in CORT, STR, or HIP.	NA	NA	No significant changes in NE in CORT, STR, or HIP.	Bai et al., 1999
2,5-bis(glutathion-S-yl)-alphamethyldopamine	150 nmol X 4, every 12 hrs	intrastriatal	Decrease in CORT. No significant changes in STR, HIP, MID/DI/TEL and PONS.	Decrease in CORT. No significant changes in STR, HIP, MID/DI/TEL and PONS.	NA	No significant changes in CORT, STR, or HIP.	NA	NA	No significant changes in NE in CORT, STR, or HIP.	Bai et al., 1999
2,5-bis(glutathion-S-yl)-alphamethyldopamine	300 nmol X 4, every 12 hrs	intrastriatal	Decreased in CORT and STR. No significant change in HIP, MID/DI/TEL, and PONS.	Decreased in CORT and STR; Increased in HIP. No significant change in MID/DI/TEL and PONS.	NA	No significant changes in CORT, STR, or HIP.	NA	NA	No significant changes in NE in CORT, STR, or HIP.	Bai et al., 1999
5-(glutathion-S-yl)-alphamethyldopamine	200 nmol X 4, every 12 hrs	intracortical	Decreased in CORT. No significant changes in STR, HIP, MID/DI/TEL, and PONS.	No significant change in STR, CORT, HIP, MID/DI/TEL, and PONS.	NA	No significant changes in CORT, STR, or HIP.	NA	NA	No significant changes in NE in CORT, STR, or HIP.	Bai et al., 1999

Table 4.1 (continued). Studies of the Neurotoxicity of Putative MDMA Metabolites

PUTATIVE METABOLITE	DOSE	ROUTE	5HT	5HIAA	TPH	DA	TH	DOPAC/HVA	OTHER CHANGES	REFERENCE
5-(glutathion-S-yl)-alphamethyldopamine	400 nmol X 4, every 12 hrs	intracortical	Decreased in CORT and STR. No significant change in HIP, MID/DI/TEL and PONS.	No significant change in CORT, STR, HIP, MID/DI/TEL and PONS.	NA	No significant changes in CORT, STR, or HIP.	NA	NA	No significant changes in NE in CORT, STR, or HIP.	Bai et al., 1999
5-(glutathion-S-yl)-alphamethyldopamine	200 nmol X 4, every 12 hrs	intrastriatal	Decreased in CORT and STR. No significant changes in HIP, MID/DI/TEL and PONS.	No significant changes in CORT, HIP, STR, MID/DI/TEL and PONS .	NA	No significant changes in CORT, STR, or HIP.	NA	NA	No significant changes in NE in CORT, STR, or HIP.	Bai et al., 1999
5-(glutathion-S-yl)-alphamethyldopamine	400 nmol X 4, every 12 hrs	intrastriatal	Decreased in STR. No significant changes in HIP, CORT, MID/DI/TEL and PONS.	Increase in STR. No significant change in CORT, HIP, MID/DI/TEL and PONS.	NA	No significant changes in CORT, STR, or HIP.	NA	NA	No significant changes in NE in CORT, STR, or HIP.	Bai et al., 1999
5-(N-acetylcystein-S-yl)-alphamethyldopamine	7 & 20 nmol X 4, every 12 hrs	intrastriatal	Decreased in STR. No significant change in CORT, HIP, MID/DI/TEL, PONS.	Decreased in STR. No significant change in CORT, HIP, MID/DI/TEL and PONS.	NA	No significant changes in CORT, STR, or HIP.	NA	NA	No significant change in NE in CORT, STR, or HIP.	Bai et al., 1999
5-(N-acetylcystein-S-yl)-alphamethyldopamine	7 & 20 nmol X 4, every 12 hrs	intracortical	Decreased in CORT. No significant change in STR, HIP, MID/DI/TEL, and PONS.	Decreased in CORT. No significant change in STR, HIP, MID/DI/TEL and PONS.	NA	No significant changes in CORT, STR, or HIP.	NA	NA	No significant change in NE in CORT, STR, or HIP.	Bai et al., 1999

Table 4.1 (continued). Studies of the Neurotoxicity of Putative MDMA Metabolites

PUTATIVE METABOLITE	DOSE	ROUTE	5HT	5HIAA	TPH	DA	TH	DOPAC/HVA	OTHER CHANGES	REFERENCE
5-(N-acetylcystein-S-yl)-alphamethyldopamine	7 & 20 nmol X 4, every 12 hrs	intrahippocampal	For HIP, no significant change with dose of 7 nmol, but a decrease at dose of 20 nmol. No significant change in CORT, STR, MID/DI/TEL, and PONS.	No change in CORT, STR, MID/DI/TEL, and PONS. In an apparent oversight by the authors, there is no mention of whether or not there is a change in the HIP.	NA	No significant changes in CORT, STR, or HIP.	NA	NA	No significant change in NE in CORT, STR, or HIP.	Bai et al., 1999
5-(glutathion-S-yl)-alphamethyldopamine	720 nmol X 4, every 12 hrs	ICV	No significant changes in CORT, STR, or HIP.	NA	NA	NA	NA	NA	NA	Miller et al., 1997
5-(N-acetylcystein-S-yl)-alphamethyldopamine	100 nmol X 4, every 12 hrs	ICV	No significant changes in CORT, STR, or HIP.	NA	NA	NA	NA	NA	NA	Miller et al., 1997
2,5-bis(glutathion-S-yl)-alphamethyldopamine	475 nmol X 4, every 12 hrs	ICV	Decreased in ipsilateral CORT and HIP not STR. Decreased in contralateral CORT not STR or HIP. No change in PONS and midbrain.	Decreased in ipsilateral HIP not STR and CORT. No change in contralateral CORT, STR, or HIP.	NA	No change in STR.	NA	No change in DOPAC or HVA in STR.	NA	Miller et al., 1997
5-(glutathion-S-yl)-alphamethyldopamine	720 nmol X 4, every 12 hrs	ICV	No significant change in MID/DI/TEL, CORT, STR, HIP.	No significant change in MID/DI/TEL, CORT, STR, HIP.	NA	No significant change in STR, MID/DI/TEL, PONS/MED, or HYPO.	NA	No change in DOPAC or HVA in STR, MID/DI/TEL, PONS/MED, or HYPO.	No change in NE in HYPO, MID/DI/TEL, or PONS/MED.	Miller et al., 1996

Table 4.1 (continued). Studies of the Neurotoxicity of Putative MDMA Metabolites

PUTATIVE METABOLITE	DOSE	ROUTE	5HT	5HIAA	TPH	DA	TH	DOPAC/HVA	OTHER CHANGES	REFERENCE
2,4,5-trihydroxtamphetamine	0.25 umol	ICV	Decreased in HIP not STR.	Decreased in HIP not STR.	Decreased in HIP and STR.	Decreased in STR.	Decreased in SN.	Decreased DOPAC in STR. HVA normal in STR.	Decreased NE in HIP.	Elayan et al., 1992
2,4,5-trihydroxtamphetamine	0.5 umol	ICV	Decreased in HIP not STR.	Decreased in HIP not STR.	Decreased in HIP and STR.	Decreased in STR.	Decreased in SN.	Decreased DOPAC and HVA in STR.	Decreased NE in HIP.	Elayan et al., 1992
6-hydroxy-MDMA	1 umol	ICV	NA	NA	No change in HIP and STR.	NA	No change in STR or SN.	NA	No changes in NE in HIP.	Elayan et al., 1992
6-hydroxy-MDA	1 umol	ICV	NA	NA	Increased in STR not HIP.	NA	Increased in SN not STR	NA	No changes in NE in HIP.	Elayan et al., 1992
3,4-dihydroxymethamphetamine (alpha-methylepinine)	135 ug	ICV	NA	NA	No change in STR, HIP, or frontal CORT.	NA	Increased in STR.	NA	NA	Elayan et al., 1992
3,4-dihydroxymethamphetamine (alpha-methylepinine)	300 ug	ICV	No change in HYPO, CORT, HIP, or STR.	No change in HYPO, CORT, HIP, or STR.	NA	No change in HYPO, CORT, or STR.	NA	No change in DOPAC or HVA in CORT or STR.	No change in NE in HYPO, CORT, HIP, or STR.	Steele et al., 1991
3,4-dihydroxymethamphetamine (alpha-methylepinine)	600 ug	ICV	No change in HYPO, CORT, HIP, or STR.	Increase in HIP. No change in HYPO, CORT, or STR.	NA	No change in HYPO, CORT, or STR.	NA	No change in DOPAC or HVA in CORT or STR.	No change in NE in HYPO, CORT, HIP, or STR.	Steele et al., 1991

Table 4.1 (continued). Studies of the Neurotoxicity of Putative MDMA Metabolites

PUTATIVE METABOLITE	DOSE	ROUTE	5HT	5HIAA	TPH	DA	TH	DOPAC/HVA	OTHER CHANGES	REFERENCE
2,4,5-trihydroxymethamphetamine	100 ug	ICV	Decreased in HIP not STR or FCx.	No change in FCx, HIP, or STR.	Decreased in HIP, STR. Increased in DR. No change in FCx or MR.	Decreased in STR.	Decreased in STR not SN.	Decreased DOPAC and HVA in STR.	NA	Johnson et al., 1992
2,4,5-trihydroxymethamphetamine	200 ug	ICV	Decreased in HIP, STR, and FCx.	No change in FCx, HIP, or STR.	Decreased in HIP, STR. Increased in DR. No change in FCx or MR.	Decreased in STR.	Decreased in STR not SN.	Decreased DOPAC and HVA in STR.	NA	Johnson et al., 1992
6-hydroxy-MDMA	10 mg/kg	IP	No change in HIP or CORT.	NA	NA	NA	NA	NA	NA	Zhao et al., 1992
6-hydroxy-MDMA	20 mg/kg	IP	No change in HIP or CORT.	NA	NA	No change in STR.	NA	NA	NA	Zhao et al., 1992
6-hydroxy-MDMA	100 ug	ICV	No change in HIP or CORT.	NA	NA	NA	NA	NA	NA	Zhao et al., 1992
6-hydroxy-MDMA	400 ug	ICV	No change in HIP or CORT.	NA	NA	No change in STR.	NA	NA	NA	Zhao et al., 1992
6-hydroxy-MDMA	?	intrastriatal	No change in STR.	NA	NA	No change in STR.	NA	NA	NA	Zhao et al., 1992
2,4,5-trihydroxymethamphetamine	100 ug	ICV	No change in STR.	NA	NA	Decreased in STR.	NA	NA	NA	Zhao et al., 1992
2,4,5 trihydroxymethamphetamine	50 ug	intrastriatal	Decreased in STR.	NA	NA	Decreased in STR.	NA	NA	NA	Zhao et al., 1992

Table 4.1 (continued). Studies of the Neurotoxicity of Putative MDMA Metabolites

PUTATIVE METABOLITE	DOSE	ROUTE	5HT	5HIAA	TPH	DA	TH	DOPAC/HVA	OTHER CHANGES	REFERENCE
2,4,5-trihydroxymethamphetamine	100 ug	intrastriatal	Decreased in STR.	NA	NA	Decreased in STR.	NA	NA	NA	Zhao et al., 1992
2,4,5-trihydroxymethamphetamine	100 ug	intracortical	No change in CORT.	NA	NA	NA	NA	NA	NA	Zhao et al., 1992
2,4,5-trihydroxymethamphetamine	400 ug	intracortical	Decreased in CORT.	NA	NA	NA	NA	NA	NA	Zhao et al., 1992
alpha-methyldopamine	400 ug	intrastriatal	No change in STR.	NA	NA	NA	NA	NA	NA	McCann & Ricaurte, 1991
alpha-methyldopamine (50 mg/kg pargyline ip pretreatment 30-45 min pre)	400 ug	ICV	No change in STR.	NA	NA	NA	NA	NA	NA	McCann & Ricaurte, 1991
3-methoxy-4-hydroxy-amphetamine (50 mg/kg pargyline ip pretreatment 30-45 min pre)	400 ug	ICV	No change in STR.	NA	NA	NA	NA	NA	NA	McCann & Ricaurte, 1991
alpha-methyl-dopa (25 mg/kg carbidopa pretreatment)	200 mg/kg, twice daily for 4 days	sc	No change in STR.	NA	NA	NA	NA	NA	NA	McCann & Ricaurte, 1991
3-O-methyl-alpha-methyl-dopa (25 mg/kg carbidopa pretreatment)	200 mg/kg, twice daily for 4 days	sc	No change in STR.	NA	NA	NA	NA	NA	NA	McCann & Ricaurte, 1991
simulataneous 3-O-methyl-alpha-methyl-dopa and alpha-methyl-dopa (25 mg/kg carbidopa pretreatment)	200 mg/kg each drug, twice daily for 4 days	sc	No change in STR.	NA	NA	NA	NA	NA	NA	McCann & Ricaurte, 1991

presence of MDMA, when the metabolite has high concentrations in the brain for several hours, or when certain acute effects of MDMA have already occurred. In such situations, administering the toxic metabolite on its own would not necessarily lead to toxicity. Thus, it is hard to interpret the many studies in which an MDMA metabolite was administered and no evidence of neurotoxicity was found (Elayan et al. 1992; Johnson et al. 1992a; McCann and Ricaurte 1991; Steele et al. 1991; Zhao et al. 1992). One can also investigate the potential role of metabolites by altering the MDMA metabolism and determining whether that alters neurotoxicity. Thus far, attempts to alter MDMA metabolism in rats with SKF-525A or phenobarbital have not provided evidence that MDMA metabolites are important in neurotoxicity (Gollamudi et al. 1989). Studies examining the neurotoxicity of centrally administered MDMA metabolites are summarized in **Table 4.1**.

A number of studies have focused on the MDMA metabolites, 3,4-dihydroxymethamphetamine (DHMA, also called alpha-methylepinine) and 3,4-dihydroxyamphetamine (DHA, also called alpha-methyldopamine). These metabolites are readily oxidized to quinones. Spontaneous oxidation of these quinones could generate hydrogen peroxide and superoxide radicals (Horton and Fairhurst 1987). Neither DHA nor DHMA has been found to produce long-term 5HT depletions when infused into the brains of rats (Johnson et al. 1992a; McCann and Ricaurte 1991; Miller et al. 1996; Steele et al. 1991). However, DHA (and likely DHMA) may become neurotoxic after forming thioether conjugates. Conjugation is considered the second phase of drug metabolism and can be seen as an attempt by the body to make foreign compounds more polar and thus more easily excreted in urine and bile. This is accomplished by adding functional groups to foreign compounds, forming products such as sulfates, glucuronides, and peptides. In some cases, this "detoxifying" process fails and conjugates of foreign compounds can have increased toxicity in comparison to the unconjugated compounds.

Several thioether metabolites of DHA can produce selective long-term (7 day) decreases in 5HT levels when infused into rat brains (Bai et al. 1999; Miller et al. 1996; 1997; Monks et al. 1999). These 5HT depleting metabolites include 2,5-bis-(Glutathion-S-yl)-alpha-methyldopamine, 5-(Glutathion-S-yl)-alpha-methyldopamine, and 5-(N-acetylcystein-S-yl)-alpha-methyldopamine. While DHA and DHMA are likely too polar to cross the blood-brain barrier, their conjugates are transported across by specialized pumps. The doses of conjugated metabolites required to deplete 5HT are relatively low. If only two percent of a systemic neurotoxic dose is converted to these metabolites and reaches the brain, this may be sufficient to produce 5HT depletions (Bai et al. 1999). It remains to be seen if the 5HT depletions produced by these conjugated metabolites are accompanied by the other features of MDMA neurotoxicity, such as axonal changes and SERT decreases. If the effects of these conjugated metabolites fully mimic MDMA neurotoxicity, this would provide strong evidence that they play a significant role in this toxicity.

Dopamine Metabolites as a Source of Oxidative Stress

It has also been suggested that some of the dopamine released by MDMA may be transported by SERT into serotonergic axons (Faraj et al. 1994) and subsequently oxidized (Nash 1990; Schmidt and Kehne 1990; Sprague and Nichols 1995b). The oxidation of dopamine can form

hydrogen peroxide, which, in turn, may produce hydroxyl radicals. A quinone-like dopamine metabolite may also be formed with potential to generate further free radicals (Cadet and Brannock 1998; Graham et al. 1978). Among many other potential toxic effects on cells, dopamine oxidation products have been shown to impair mitochondrial functioning (Berman and Hastings 1999).

There is some evidence for dopaminergic involvement in MDMA neurotoxicity. Previous dopaminergic neurotoxicity in the substantia nigra reduces subsequent MDMA neurotoxicity (Schmidt et al. 1990c; Stone et al. 1988). Coadministration of the dopamine reuptake inhibitor, mazindol, reduces MDMA-induced striatal dopamine release and hydroxyl radical formation as well as the long-term serotonin depletion (Nash and Brodkin 1991; Shankaran et al. 1999b). This attenuation of the effects of MDMA takes place without altering the acute hyperthermic response to MDMA. The neuroprotective effect of mazindol is consistent with reports that MDMA neurotoxicity is inhibited by drugs that suppress MDMA-induced dopamine release, such as GBR-12909 (Stone et al. 1988).

Over the last few years, some of the evidence for dopaminergic involvement in MDMA neurotoxicity has been reinterpreted. A number of dopaminergic drugs that modify MDMA neurotoxicity probably act by modulating body temperature rather than through a specifically dopaminergic mechanism, as previously thought. These drugs include alpha-methyl-para-tyrosine (Malberg et al. 1996) and haloperidol (Colado et al. 1999c). The mechanism by which L-dopa increases neurotoxicity is currently unclear with conflicting findings from two groups (Colado et al. 1999c; Schmidt et al. 1991). Although the monoamine oxidase-B (MAO-B) inhibitors, L-Deprenyl (selegiline) and MDL-72974, each attenuate MDMA neurotoxicity (Sprague and Nichols 1995a; b), they may act by scavenging free radicals since blockade of MAO-B gene expression with antisense oligonucleotides is not neuroprotective (Sprague et al. 1999).

If dopamine plays a central role in MDMA neurotoxicity, one might expect a correlation between dopamine release and MDMA neurotoxicity. Nichols and colleagues found a linear correlation between the acute striatal dopamine release produced by a series of different substituted amphetamines and subsequent long-term 5HT depletions (Johnson et al. 1991; Nash and Nichols 1991). However, another group (Colado et al. 1999c) reported that non-neurotoxic and neurotoxic doses of MDMA produced comparable amounts of dopamine release in rats. Furthermore, some brain areas with relatively little dopamine such as the hippocampus show profound 5HT depletions after MDMA. In fact, Shankaran and Gudelsky (1998) reported that blocking MDMA-induced acute dopamine release in the hippocampus (using the norepinephrine uptake inhibitor, desipramine) did not prevent long-term 5HT depletions in that region. The same report suggested that the mechanism of MDMA neurotoxicity may vary between brain regions.

In conclusion, dopamine release seems to play a role in MDMA neurotoxicity, but there is currently no direct evidence that a metabolite of dopamine acts as a toxin after MDMA administration.

There is Currently Little Evidence that 5HT Metabolites Act as Toxins

Berger et al. (1992a) suggested that 5HT metabolites may be responsible for phenethylamine-induced neurotoxicity. Indeed, hydroxylated metabolites of 5HT such as 5,6- and 5,7-dihydroxytryptamine are selective serotonergic neurotoxins. More recently, Dryhurst and colleagues have demonstrated that oxidation of 5HT by superoxide anion radical can lead to tryptamine-4,5-dione, a mitochondrial toxin (Jiang et al. 1999; Wrona and Dryhurst 1998).

Although such 5HT metabolites can be neurotoxic, there is currently no evidence to suggest these specific metabolites contribute to MDMA neurotoxicity. Toxic 5HT metabolites have not yet been reported in brains of MDMA-treated animals. Although a molecule resembling 5,6-dihydroxytryptamine was reported by Commins et al. (1987a) in rat brains after neurotoxic regimens of both parachloroamphetamine and methamphetamine, this has not been confirmed. On the contrary, a more stable product of hydroxyl-mediated 5HT oxidation, 5-(hydroxy-indoyl)-3-(ethylamino)-2-oxindole, is not elevated in rat brains after neurotoxic regimens of methamphetamine (Yang et al. 1997). As evidence against the possibility of neurotoxic 5HT metabolites, Sprague et al. (1994) found that pretreatment with the 5HT precursors, tryptophan or 5-hydroxytryptophan, decreased MDMA neurotoxicity. This finding is somewhat unexpected given that 5-hydroxytryptophan pretreatment enhances MDMA-induced 5HT and dopamine release in the striatum (Gudelsky and Nash 1996). In addition, prior depletion of 5HT with parachlorophenylalanine does not decrease neurotoxicity (Brodkin et al. 1993). Thus, increasing 5HT is protective, while decreasing 5HT is not. It is difficult to reconcile these findings with the possibility of neurotoxic 5HT metabolites.

Glutamate Does Not Appear to Play a Major Role in MDMA neurotoxicity

Excitatory amino acids such as glutamate have well-established potential to damage neurons (Choi 1992; Olney 1994). A role for glutamate in MDMA neurotoxicity was suggested by a report that the N-methyl-D-aspartate (NMDA) antagonist, dextrorphan, inhibited MDMA-induced 5HT depletions in the rat striatum (Finnegan et al. 1990). Subsequent studies employing other NMDA antagonists, such as dizocilpine (also called MK-801), have not supported this conclusion. Although it is neuroprotective, dizocilpine appears to protect against MDMA neurotoxicity through a thermoregulatory mechanism (Farfel and Seiden 1995). Glutamate antagonists that do not block MDMA-induced hyperthermia are not neuroprotective (Colado et al. 1998; Farfel and Seiden 1995). As further evidence against a role for glutamate in MDMA neurotoxicity, Nash and Yamamoto (1992) reported that a neurotoxic MDMA regimen had no effect on acute glutamate efflux in the striatum of rats. Finally, excitotoxicity does not usually produce selective axon loss. Thus, there currently appears to be no strong evidence that glutamate plays in role in the mechanism of MDMA neurotoxicity.

A Possible Role for Ca^{2+} in MDMA Neurotoxicity

Despite the lack of evidence that glutamate plays a role in MDMA neurotoxicity, there may be similarities in the intracellular mechanisms of excitotoxicity and MDMA neurotoxicity. Excitotoxicity involves increases in Ca^{2+} influx into the cell. MDMA may also disturb the cellular homeostasis of serotonergic axons by increasing intracellular Ca^{2+} concentrations. Kramer, Poblete, and Azmitia (1998) reported that MDMA produces a Ca^{2+} dependent protein kinase C translocation through its interactions with the SERT. They suggested that sustained interaction of MDMA with the SERT may therefore increase intracellular Ca^{2+} concentrations. High intracellular Ca^{2+} concentrations could impair cellular functioning in a number of ways. Increased intracellular Ca^{2+} may impair mitochondrial activity since elevated levels of Ca^{2+} have been found to increase free radical production in isolated cerebellar and cerebral mitochondria (Dykens 1994).

Studies employing calcium channel blockers have found mixed evidence that Ca^{2+} mediates MDMA neurotoxicity. The calcium channel blocker, flunarazine, protects against the MDMA-induced 5HT depletions (Finnegan et al. 1993) and reductions in cortical and nigrostriatal TPH activity (Johnson et al. 1992b). However, other L-type calcium channel antagonists, such as nimodipine, are not protective (Johnson et al. 1992b). These studies did not control for possible effects of drug treatment on body temperature.

Increased intracellular Ca^{2+} could activate Ca^{2+} / calmodulin-dependent nitric oxide synthase (NOS). Resulting nitric oxide (NO) can react with the superoxide anion to form peroxynitrite, a highly reactive free radical. NO also impairs mitochondrial activity through poorly understood mechanisms (Brorson et al. 1999). Increased NOS activity and resulting excessive NO levels may therefore participate in MDMA neurotoxicity, although evidence for this is ambiguous. The NO donor nitroprusside increased MDMA-induced cytotoxicity in an *in vitro* study using cultured human serotonergic cells (Simantov and Tauber 1997). Brain NOS activity is increased in the frontal and parietal cortices 6 hrs after a neurotoxic regimen of MDMA in rats (Zheng and Laverty 1998). Pretreatment with the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) decreases MDMA neurotoxicity in those areas. On the other hand, L-NAME also induces hypothermia and other NOS inhibitors lacking this hypothermic effect are not protective (Taraska and Finnegan 1997).

Extent of Neurotoxicity Depends on Dose, Route of Administration, Animal Age, and Species.

Neurotoxicity is dose-dependent. Long-term changes occur in rats at doses approximately 5 to 10 times higher than those known to be psychoactive. A study using male Dark Agouti rats (O'Shea et al. 1998), found that, seven days after drug exposure, 4 mg/kg intraperitoneally injected MDMA did not affect hippocampal 5HT levels. 10 mg/kg MDMA decreased hippocampal 5HT to about 65% of control levels, while 15 mg/kg MDMA lowered levels to about 40% of controls. Cortical SERT density was similarly decreased, showing that changes were not just due to altered synthesis of 5HT. The 5HT-depleting effects of 0, 20, or 40 mg/kg

subcutaneous MDMA were compared at two and eight weeks after drug administration in Sprague-Dawley rats (Commins et al. 1987b). Interestingly, doubling the dose from 20 to 40 mg/kg only moderately increased the extent of 5HT depletions. For example, 5HT levels in the hippocampus at post 8 weeks were reduced to 70% of control levels by 20 mg/kg MDMA and 60% of control levels by 40 mg/kg MDMA.

Single vs. Multiple Dose Exposures. Most MDMA neurotoxicity studies have used multiple dose regimens. These studies show that "binge" use of MDMA carries greater risk of neurotoxicity than single doses. When administered repeatedly, a non-neurotoxic dose of MDMA can become neurotoxic (Battaglia et al. 1988a; O'Shea et al. 1998). Multiple dose neurotoxic regimens appear able to produce more profound and possibly more lasting serotonergic changes than single MDMA administration (Battaglia et al. 1988b). The results of multiple dose studies are difficult to compare across species since the same interdosing interval can have very different effects in two species with different clearance rates of MDMA.

Route of Administration. The importance of route of administration in altering long-term serotonergic changes has been investigated. In the rat, subcutaneous injection and oral administration of MDMA produce comparable 5HT depletions in the hippocampus (Finnegan et al. 1988). Studies with nonhuman primates have yielded less consistent results. In the squirrel monkey, repeated oral administration of MDMA resulted in only one-half to two-thirds as much 5HT depletion as the equivalent subcutaneous dose (Ricaurte et al. 1988a). In the rhesus monkey, in contrast, repeated oral administration of MDMA produced twice the decrease in hippocampal SERT activity as was produced by repeated subcutaneous injection (Kleven et al. 1989). These apparent differences between nonhuman primate species increase the difficulty of assessing the risk of oral MDMA administration in humans.

Age of Animal. Although young rats undergo MDMA-induced acute serotonergic effects (Broening et al. 1994), they are insensitive to both MDMA-induced hyperthermia (Broening et al. 1995) and MDMA neurotoxicity. Rats appear to become vulnerable to MDMA neurotoxicity at about 35 days after birth, possibly due to changes in the dopaminergic system (Aguirre et al. 1998b). However, Broening et al. (2001) were able to produce small serotonin depletions in newborn rats using 5 mg/kg MDMA subcutaneously injected twice daily for 10 days. This shows that newborn rats are resistant, but not invulnerable, to MDMA neurotoxicity. Few studies have examined whether aged animals have increased vulnerability to MDMA neurotoxicity. Young adult (3 month old) and aged (24 months old) mice undergo comparable dopaminergic neurotoxicity, but display age-specific differences in accompanying changes in neurotransmitter turnover and cell signaling activity (Slotkin et al. 2000). These differences were interpreted by the researchers as indicating that aged mice were less able to compensate on the cellular level for loss of dopaminergic axons.

Species and Strain Differences in Vulnerability

A variety of species have been used MDMA neurotoxicity research. These species include mice, rats, guinea pigs (Battaglia et al. 1988b; Commins et al. 1987b), cynomolgus monkeys (Ricaurte et al. 1988c; Wilson et al. 1989), baboons (Scheffel et al. 1998), squirrel monkeys (Fischer et al.

1995; Hatzidimitriou et al. 1999; Kleven et al. 1989; Ricaurte 1989; Ricaurte et al. 1988a; Ricaurte et al. 1988b; Ricaurte et al. 1988c; Ricaurte et al. 1992), and rhesus monkeys (Ali et al. 1993; Beardsley et al. 1986; De Souza et al. 1990; Frederick et al. 1998; Frederick et al. 1995; Insel et al. 1989; Jagust et al. 1996; Ricaurte et al. 1988c; Slikker et al. 1988; Slikker et al. 1989; Taffe et al. 2001; Wilson et al. 1989). All species tested are vulnerable to MDMA neurotoxicity. However, mice are unusual in that they primarily undergo long-term dopaminergic, rather than serotonergic, changes. There appear to be species differences in the MDMA exposure required to produce neurotoxicity and the extent of depletions produced. However, there are insufficient data from most species to indicate the species-specific dose-response function.

Strain Differences in Vulnerability. Rat strains differ in sensitivity to MDMA neurotoxicity. For example, neurotoxicity could not be detected when 25 mg/kg MDMA was intraperitoneally administered to randomly bred albino rats (Logan et al. 1988). In contrast, Dark Agouti rats have a threshold between 4 and 10 mg/kg intraperitoneally injected MDMA for undergoing 5HT depletions (O'Shea et al. 1998). These apparent strain differences may also be influenced by differences in ambient temperature and animal housing (Dafters 1995; Gordon and Fogelson 1994).

Primates are More Vulnerable than Rats. In comparison to rats, nonhuman primates seem to have a lower threshold dose for MDMA neurotoxicity and generally undergo more extensive decreases in serotonergic markers (Ali et al. 1993; Fischer et al. 1995; Insel et al. 1989; Ricaurte et al. 1992; Ricaurte and McCann 1992; but see also De Souza et al. 1990 for slightly different results). This has suggested to some that humans may be even more sensitive than nonhuman primates. Possible reasons for the increased vulnerability of nonhuman primates compared to rodents include the increased length of serotonergic axons in primates, the increased degree of axonal myelination in primates, and expected pharmacokinetic differences between species (Campbell 1995; Fischer et al. 1995). Research with fenfluramine suggests that species differences in fenfluramine-induced serotonergic neurotoxicity may be largely due to pharmacokinetic differences (Mennini et al. 1996). However, no published studies have documented the pharmacokinetics of MDMA in nonhuman primates.

Squirrel Monkey Research. Many MDMA neurotoxicity studies have used squirrel monkeys as subjects (Fischer et al. 1995; Hatzidimitriou et al. 1999; Kleven et al. 1989; Ricaurte 1989; Ricaurte et al. 1988a; Ricaurte et al. 1988b; Ricaurte et al. 1988c; Ricaurte et al. 1992). The threshold dose for producing long-term 5HT depletions in squirrel monkeys is between 2.5 and 5 mg/kg oral MDMA. Two weeks after a single 5.0 mg/kg oral MDMA dose to this species, 5HT levels were decreased to 83% of control levels in the hypothalamus and 79% of controls in the thalamus but were not changed in other examined brain regions (Ricaurte et al. 1988a). SERT density was not reported in this study. In contrast, no long-term serotonergic changes occurred after 2.5 mg/kg MDMA was given orally every two weeks for four months to squirrel monkeys (Ricaurte, unpublished, cited in Vollenweider et al. 1999a).

Rhesus Monkey Research. Another commonly studied nonhuman primate species is the rhesus monkey (Ali et al. 1993; De Souza et al. 1990; Frederick et al. 1998; Frederick et al. 1995; Insel

et al. 1989; Jagust et al. 1996; Ricaurte et al. 1988c; Slikker et al. 1988; Slikker et al. 1989; Taffe et al. 2001; Wilson et al. 1989). Determining the threshold dose for 5HT depletions in this species is difficult since all published studies using rhesus monkeys have employed multiple dose neurotoxic regimens. In one study, 1.25 mg/kg oral MDMA did not produce any long-term serotonergic changes when given twice daily for 4 consecutive days. Similarly repeated doses of 2.5 mg/kg MDMA lowered hippocampal 5HT (to about 80% of controls) but did not affect levels in 6 other brain regions at post 1 month (Ali et al. 1993). Another experiment (Insel et al. 1989) found that 2.5 mg/kg MDMA given intramuscularly twice daily for 4 days to rhesus monkeys produced extensive (possibly short term) 5HT depletions but did not alter SERT density at 16 to 18 hours after the last drug exposure. Since SERT was unaffected, the researchers concluded that axonal loss had not occurred, despite the (possibly short-term) 5HT depletions. One study, which raised interesting questions about possible tolerance to MDMA neurotoxicity, investigated the long-term effects of escalating doses of MDMA (Frederick et al. 1995). Intramuscular MDMA (0.10-20.0 mg/kg) was given twice daily for 14 consecutive days at each dose level and followed by three dose-response regimens using single MDMA doses up to 5.6 mg/kg. One month after the final dose-response determination and 21 months after the initial escalating dose regimen, animals were sacrificed. Few significant serotonergic effects were found. MDMA exposure did not produce significant 5HT depletions in any brain region and decreased SERT to about 60% of control levels only in the hippocampus (and not two other brain regions). Because escalating dose regimens of *d*-fenfluramine (Caccia et al. 1992) or *d*-amphetamine (Robinson and Camp 1987) provide protection against the neurotoxicity of these drugs, it seems likely that the same poorly-understood phenomenon is being detected here. Thus, data on rhesus monkeys are complex and perhaps all that can be said with certainty is that the threshold dose for long-term 5HT depletions appears to be above 1.25 mg/kg oral MDMA in this species.

Why Are Such High Doses Used In Neurotoxicity Research?

Research on MDMA neurotoxicity has sometimes been criticized for the high, repeated dose regimens that are commonly used. Some have questioned whether repeated injections of 20 mg/kg MDMA in rodents can provide useful information about the toxicity of single oral doses of 1.7 to 2.0 mg/kg MDMA in humans. It is true that many of the neurotoxic regimens are not designed to be clinically relevant but were intended to maximize the serotonergic neurotoxicity of MDMA in order to better understand its mechanisms and consequences.

However, comparing dose on the basis on body weight can be misleading. In general, smaller species excrete drugs more quickly and form metabolites in greater amounts than larger species. This is due to many factors including the proportionally larger livers and kidneys and faster blood circulation times in smaller mammals (Lin 1998; Mordenti and Chappell 1989). As a result of such factors, the time it takes to lower the plasma levels of MDMA by half is about 1.5 hours in a rat (Cho et al. 1990) and about 8 hours in a human (Mas et al. 1999). This suggests that small species may require higher doses (in mg/kg) to achieve drug exposures comparable to those seen in larger species. These considerations at least partially justify the apparently high doses commonly used in rodent toxicity studies. Unfortunately, higher doses tend to alter the character of the drug exposure. While they lengthen the time smaller animals are exposed to the

drug, they also tend the produce higher peak blood concentrations of drug and greater acute effects than occur in larger species at lower doses.

A number of techniques have been developed for estimating equivalent drug doses in different species (useful articles from this large literature include Ings 1990; Lin 1998; Mahmood 1999; Mordenti and Chappell 1989). One of the most commonly used techniques, allometric interspecies scaling, involves administering a drug to different species and measuring resulting blood concentrations of drug. These measurements are then used to empirically determine the relationships between species weight, drug exposure, and dose. Drug exposure in humans can be estimated from these relationships. In these estimates, equivalent drug exposures are assumed to produce equivalent drug effects, including neurotoxicity. Ricaurte and colleages (2000) recently estimated that as little as 1.28 mg/kg MDMA may produce long-term 5HT depletions in humans if interspecies dose conversions for MDMA follow a pattern that is common for drugs that are not extensively metabolized. Estimates of this sort are useful for emphasizing that the MDMA dose required to produce neurotoxicity in humans may be within the range of commonly administered doses, despite the seemingly higher doses used in rodent studies.

However, such estimates require making assumptions about the mechanisms of neurotoxicity. For example, it is necessary to assume that the different species experience comparable drug effects when blood concentrations of drug are the same. This may not be true of neurotoxicity. Neuronal responses to focal ischemic/reperfusion injury show temporal differences between rats and baboons (Tagaya et al. 1997). Several other possible reasons for species differences in MDMA neurotoxicity have already been given in the paragraph on differences in vulnerabilities between the primate and the rat. In addition, species may differ in the brain concentration of drug produced by a given blood concentration. It is not known if this is the case with MDMA, although it does seem to be true for fenfluramine (Campbell 1995). In rats, MDMA concentrations in the brain are 7 to 10 times higher than in plasma (Chu et al. 1996). In a human fatality, postmortem MDMA concentrations were about 6 times higher in the brain than in the plasma (Rohrig and Prouty 1992), although postmortem drug redistribution may have occurred. If these data are reliable, rats may have similar peak brain levels to humans when plasma levels are the same.

Furthermore, if MDMA neurotoxicity is caused by a toxic metabolite, as some have suggested, then the more extensive metabolism of MDMA expected in smaller animals will lead to increased neurotoxicity. Formation of specific drug metabolites in different species is difficult to predict and few data are available on MDMA. Research on species differences in fenfluramine metabolism have led some to conclude that no nonhuman species provides a good model of possible human fenfluramine neurotoxicity (Caccia et al. 1995; Marchant et al. 1992). Because current data suggest that both MDMA and metabolite exposure may mediate neurotoxicity, more data are needed from more species before interspecies dose conversions can be made with any confidence.

Data from clinical MDMA studies show that there is a complex relationship between MDMA dose and blood levels of the drug and its metabolites (de la Torre et al. 2000a; Mas et al. 1999).

It appears that MDMA inactivates one of the enzymes that is important to its metabolism (an enzyme known as cytochrome p450 isozyme 2D6 or 'CYP 2D6') (Brady et al. 1986; Wu et al. 1997). As a result, small increases in dose can lead to large increases in drug exposure. When dose was increased from 125 mg to 150 mg, drug exposure (measured as area under the MDMA plasma concentration verses time curve) almost doubled in human volunteers (de la Torre et al. 2000a). At the same time, formation of some metabolites remained approximately constant. These complex dose-dependent pharmacokinetics in humans further increase the difficulty of estimating dose conversions between species. Nonetheless, these human studies with MDMA do suggest that doses above 125 mg may be associated with unexpectedly increased drug exposure and risks of acute toxicity.

Extent of Neurotoxicity in Rats is Influenced by Environment, Especially Ambient Temperature

A number of studies have explored the relationships between environmental temperature, animal core temperature, and neurotoxicity. In rats, MDMA can dose-dependently induce thermoregulatory impairment (Broening et al. 1995; Colado and Green 1995; Dafters 1994; 1995; Gordon et al. 1991) perhaps through alterations in hypothalamic functioning and drug-induced impairment in thermoregulatory behaviors. Resulting changes in animal temperature can alter neurotoxicity with hyperthermia increasing and hypothermia decreasing serotonergic depletions. Thus, degree of hyperthermia has been found to correlate with both long-term 5HT depletions in adult rats (Broening et al. 1995; Colado and Green 1995; Colado et al. 1993; Malberg and Seiden 1998) and long-term dopamine depletions in mice (Miller and O'Callaghan 1994). In addition to the ambient temperature, the degree of hyperthermia is influenced by the thermal conductivity of animal housing and animal hydration status (Dafters 1995; Gordon and Fogelson 1994).

The mechanisms by which temperature affects MDMA neurotoxicity are unclear. Plasma levels of MDMA in rats (Colado and Green 1995) and brain levels of MDMA in mice (Campbell 1996) do not appear to be influenced by changes in animal core temperature. MDMA-induced neurotransmitter release may be temperature sensitive (Sabol and Seiden 1998), although studies examining the temperature dependence of methamphetamine-induced DA release have reported conflicting findings (Bowyer et al. 1993; LaVoie and Hastings 1999). It may also be that increased temperature nonspecifically increases the rate of reactions and contributes to oxidative stress, as occurs in ischemic neurotoxicity (Globus et al. 1995). Prolonged hyperthermia has been shown to decrease mitochondrial immunoreactivity in some brain regions, suggesting decreased energy stores (Burrows and Meshul 1999). However, hyperthermia on its own does not selectively damage the serotonergic system.

Although hyperthermia increases neurotoxicity and hypothermia decreases it, MDMA neurotoxicity can occur without hyperthermia (Broening et al. 1995). In addition, the link between temperature and neurotoxicity has primarily been investigated in rodents. One study examining methamphetamine-induced neurotoxicity in vervet monkeys reported that hypothermia (induced by the NMDA antagonist dizocilpine) failed to protect against

Table 4.2. Studies of Drugs Modifying MDMA Neurotoxicity and Hyperthermia

MDMA REGIMEN (dose, route)	OTHER INTERVENTION	CATEGORY OF OTHER INTERVENTION	OUTCOME OF MDMA + OTHER INTERVENTION	HYPER-THERMIA?	REFERENCE
20 mg/kg sc, twice daily for 4 d	l-tryptophan 400 mg/kg ip twice daily for 4 d, 30 m pre MDMA	5HT precursor	Some protection	NA	(Sprague et al. 1994)
20 mg/kg sc, twice daily for 4 d	5-hydroxytryptophan 50 mg/kg ip (with RO 4-4602 50 mg/kg) twice daily for 4 d, 30 m pre MDMA	5HT precursor with a peripheral decarboxylase inhibitor	Some protection	NA	(Sprague et al. 1994)
20 mg/kg d-MDMA sc, four times, every 2 hrs	d-fenfluramine 25 mg/kg sc, 20 m before 1st and 3rd injection MDMA	5HT releaser	Some protection	Attenuated hyperthermia	(Miller and O'Callaghan 1994)
40 mg/kg sc	fluoxetine (10 mg/kg ip), 1 hr pre MDMA	5HT reuptake inhibitor	Protection	Hyperthermia	(Malberg et al. 1996)
10 mg/kg sc	DOI 2 mg/kg ip, 15 m pre MDMA	$5HT_2$ agonist	Exacerbated toxicity (5HT decrease in STR)	NA	(Gudelsky et al. 1994)
30 mg/kg MDMA HCl sc	ketanserin 5 mg/kg sc, 15 min pre MDMA	$5HT_2$ antagonist	Protection	Attenuated hyperthermina	(Aguirre et al. 1998a)
40 mg/kg sc	ketanserin (6 mg/kg ip), 1 hr pre MDMA	$5HT_2$ antagonist	Protection	Hypothermia occurred and manual warming blocked protection	(Malberg et al. 1996)
30 mg/kg sc	MDL-28133A 0.03, 0.1, 0.3, 1.0 mg/kg sc, along with MDMA	$5HT_2$ antagonist	Some protection by 0.1, 0.3, or 1.0 mg/kg but not by 0.03 mg/kg	NA	(Schmidt et al. 1992a)
30 mg/kg sc	MDL-28133A 1 mg/kg sc, along with MDMA	$5HT_2$ antagonist	Some protection	NA	(Schmidt et al. 1992a)
20 mg/kg sc	(±)-MDL 11,939 5 mg/kg sc, along with MDMA	$5HT_2$ antagonist	Some protection	NA	(Schmidt et al. 1991) also (Schmidt and Kehne 1990)
20 mg/kg sc	R-(+)-MDL 11,939 5 mg/kg sc, along with MDMA	$5HT_2$ antagonist	Some protection	NA	(Schmidt et al. 1991)
20 mg/kg sc	S-(-)-MDL 11,939 5 mg/kg sc, along with MDMA	$5HT_2$ antagonist	No protection	NA	(Schmidt et al. 1991)

Table 4.2. Studies of Drugs Modifying MDMA Neurotoxicity and Hyperthermia

MDMA REGIMEN (dose, route)	OTHER INTERVENTION	CATEGORY OF OTHER INTERVENTION	OUTCOME OF MDMA + OTHER INTERVENTION	HYPER-THERMIA?	REFERENCE
20 mg/kg sc	ritanserin 5 mg/kg sc, along with MDMA	$5HT_2$ antagonist	Some protection	NA	(Schmidt et al. 1991)
20 mg/kg sc	MDL 11,939 5 mg/kg sc, post 3 hr MDMA	$5HT_2$ antagonist	No protection	NA	(Schmidt et al. 1991)
20 mg/kg sc	MDL 11,939 5 mg/kg sc, along with MDMA	$5HT_2$ antagonist	Protection	NA	(Schmidt et al. 1990a)
20 mg/kg sc	MDL 11,939 5 mg/kg sc, post 1 hr MDMA	$5HT_2$ antagonist	Less protection than earlier intervention	NA	(Schmidt et al. 1990a)
20 mg/kg sc	MDL 11,939 5 mg/kg sc, post 3 hr MDMA	$5HT_2$ antagonist	Less protection than earlier intervention	NA	(Schmidt et al. 1990a)
20 mg/kg sc	MDL 11,939 5 mg/kg sc, post 6 hr MDMA	$5HT_2$ antagonist	No protection	NA	(Schmidt et al. 1990a)
20 mg/kg sc	MDL 11,939 5 mg/kg sc, along with MDMA	$5HT_2$ antagonist	Some protection	NA	(Schmidt et al. 1990a)
20 or 30 mg/kg sc	MDL 11,939 5 mg/kg sc, along with MDMA	$5HT_2$ antagonist	Protection	Attenuated hyperthermia	(Schmidt et al. 1990b)
20 mg/kg sc	MDL 26,508 5 mg/kg, along with MDMA	$5HT_2$ antagonist	Protection	NA	(Schmidt et al. 1990c)
20 mg/kg sc	MDL 11,939 5 mg/kg, along with MDMA	$5HT_2$ antagonist	Protection	NA	(Schmidt et al. 1990c)
20 mg/kg sc	ritanserin 1, 5 mg/kg sc, 15 m pre MDMA	$5HT_2$ antagonist	Protection	NA	(Johnson et al. 1993)
30 mg/kg sc	MDL 100,907 0.01. 0.03, 0.1, 0.3, and l mg/kg sc, along with MDMA	$5HT_2$ antagonist	Dose-dependent protection	NA	(Schmidt et al. 1992b)
30 mg/kg sc	MDL-28133A 1 mg/kg sc and L-Dopa 100 mg/kg ip (with carbidopa 25 mg/kg ip), along with (carbidopa was 30 m pre) MDMA	$5HT_2$ antagonist and DA precursor and peripheral Dopa decarboxylase inhibitor	No protection	NA	(Schmidt et al. 1992a)

Table 4.2. Studies of Drugs Modifying MDMA Neurotoxicity and Hyperthermia

MDMA REGIMEN (dose, route)	OTHER INTERVENTION	CATEGORY OF OTHER INTERVENTION	OUTCOME OF MDMA + OTHER INTERVENTION	HYPER-THERMIA?	REFERENCE
20 mg/kg ip	ondansetron 0.5 mg/kg ip, 5 m pre and 55 m post MDMA	$5HT_3$ antagonist	No protection	NA	(Colado and Green 1994)
20 mg/kg sc	MDL 73,147 5 mg/kg, along with MDMA	$5HT_3$ antagonist	No protection	NA	(Schmidt et al. 1990c)
20 mg/kg sc	prazosin 3 mg/kg, along with MDMA	Alpha-1 antagonist	No protection	NA	(Schmidt et al. 1990c)
20 mg/kg sc	chloral hydrate 400 mg/kg ip followed by 30-100 mg/kg supplements to maintain anesthesia through 3 hr post, 30 m pre MDMA	Anesthetic	Protection	Attenuated hyperthermia; high ambient temperature did not alter protection	(Schmidt et al. 1990b)
20 mg/kg sc twice, 7 hr apart	tripelennamine 20 mg/kg ip twice 7 hr apart, along with MDMA	Antihistamine; but also "moderate" hydroxyl radical scavenger.	Some protection	Attenuated hyperthermina	(Yeh et al. 1999)
20 mg/kg sc twice, 7 hr apart	pyrilamine, 20 mg/kg ip twice 7 hr apart, along with MDMA	Antihistamine; but also "potent" hydroxyl radical scavenger, SSRI, and "moderate" DA reuptake inhibitor.	Excerbated toxicity	Enhanced hyperthermia	(Yeh et al. 1999)
20 mg/kg sc twice, 7 hr apart	diphenhydramine 20 mg/kg ip twice 7 hr apart, along with MDMA	Antihistamine; but also inhibits p450 and is "moderate" DA uptake inhibitor.	Some protection	Hyperthermia	(Yeh et al. 1999)

Table 4.2. Studies of Drugs Modifying MDMA Neurotoxicity and Hyperthermia

MDMA REGIMEN (dose, route)	OTHER INTERVENTION	CATEGORY OF OTHER INTERVENTION	OUTCOME OF MDMA + OTHER INTERVENTION	HYPER-THERMIA?	REFERENCE
20 mg/kg sc twice, 6 hr apart	chlorpheniramine 10 or 25 mg/kg ip twice, 6 hr apart, along with MDMA	Antihistamine; but also inihibits 5HT uptake, inhibits p450; hydroxyl scavenger; "moderate" DA uptake inhibitor.	Protection	Hypothermia	(Yeh et al. 1999)
20 mg/kg sc twice, 7 hr apart	chlorpheniramine 20 mg/kg ip twice, 7 hr apart, along with MDMA	Antihistamine; but also inihibits 5HT uptake, inhibits p450; hydroxyl scavenger; "moderate" DA uptake inhibitor.	Protection	Hypothermia	(Yeh et al. 1999)
20 mg/kg sc twice, 7 hr apart	chlorpheniramine 20 mg/kg ip twice, 7 hr apart, 3 hrs post MDMA	Antihistamine; but also inihibits 5HT uptake, inhibits p450; hydroxyl scavenger; "moderate" DA uptake inhibitor.	Protection	Hypothermia	(Yeh et al. 1999)
20 mg/kg sc twice, 7 hr apart	chlorpheniramine 20 mg/kg ip twice, 7 hr apart, 6 hrs post MDMA	Antihistamine; but also inihibits 5HT uptake, inhibits p450; hydroxyl scavenger; "moderate" DA uptake inhibitor.	Protection	Hyperthermia until 6 hr then hypothermia	(Yeh et al. 1999)
20 mg/kg ip	alpha-lipoic acid, 100 mg/kg ip twice daily for 2 days, last injection was 30 m pre MDMA	Antioxidant	"Full" protection	Unchanged	(Aguirre et al. 1999)
10 mg/kg ip, every 2 hr for 4 injections	ascorbic acid, 100 mg/kg ip, every 2 hr for 5 injections	Antioxidant	Protection	Unchanged	(Shankaran et al. 2001)

Table 4.2. Studies of Drugs Modifying MDMA Neurotoxicity and Hyperthermia

MDMA REGIMEN (dose, route)	OTHER INTERVENTION	CATEGORY OF OTHER INTERVENTION	OUTCOME OF MDMA + OTHER INTERVENTION	HYPER-THERMIA?	REFERENCE
20 mg/kg sc	sodium ascorbate 250 mg/kg ip twice 5.5 hr apart, 30 m pre, 5 hr post MDMA	Antioxidant	Protection	NA	(Gudelsky 1996)
20 mg/kg sc	cysteine 500 mg/kg ip twice 5.5 hr apart, 30 m pre, 5 hr post MDMA	Antioxidant	Protection	NA	(Gudelsky 1996)
20 mg/kg sc	cysteine 1 g/kg, along with MDMA	Antioxidant	Protection	NA	(Schmidt and Kehne 1990)
10 mg/kg ip twice daily for 3 d	1-benzyl-piperazine 10 mg/kg ip twice daily for 3 d, along with MDMA	Benzylpiperazine derivative	No protection	NA	(Hashimoto et al. 1992b)
10 mg/kg ip twice daily for 3 d	1-piperonyl-piperazine 10 mg/kg ip twice daily for 3 d, along with MDMA	Benzylpiperazine derivative	Some protection	NA	(Hashimoto et al. 1992b)
10 mg/kg ip twice daily for 3 d	p-chlorobenzyl-piperazine 10 mg/kg ip twice daily for 3 d, along with MDMA	Benzylpiperazine derivative	Some protection	NA	(Hashimoto et al. 1992b)
10 mg/kg ip twice daily for 3 d	p-methoxy-benzyl-piperazine 10 mg/kg ip twice daily for 3 d, along with MDMA	Benzylpiperazine derivative	No protection	NA	(Hashimoto et al. 1992b)
10 mg/kg ip twice daily for 3 d	p-nitro-benzyl-piperazine 10 mg/kg ip twice daily for 3 d, along with MDMA	Benzylpiperazine derivative	Some protection	NA	(Hashimoto et al. 1992b)
10 mg/kg ip twice daily for 3 d	desipramine 10 mg/kg ip twice daily for 3 d, along with MDMA	Benzylpiperazine derivative	No protection	NA	(Hashimoto et al. 1992b)
10 mg/kg ip twice daily for 3 d	1-piperonyl-piperazine 20 mg/kg ip twice daily for 3 d, along with MDMA	Benzylpiperazine derivative, compound with 3,4-methylenedioxyphenyl group	Some protection	NA	(Hashimoto et al. 1992b)

Table 4.2. Studies of Drugs Modifying MDMA Neurotoxicity and Hyperthermia

MDMA REGIMEN (dose, route)	OTHER INTERVENTION	CATEGORY OF OTHER INTERVENTION	OUTCOME OF MDMA + OTHER INTERVENTION	HYPER-THERMIA?	REFERENCE
20 mg/kg sc	pindolol 5 mg/kg, along with MDMA	Beta-adrenoceptor antagonist and nonselective $5HT_1$ antagonist	No protection	NA	(Schmidt et al. 1990c)
10 mg/kg sc five times, every 6 hr	Flunarizine 30 mg/kg ip, 15 m pre each dose MDMA	Calcium channel blocker	Protection	NA	(Johnson et al. 1992b)
15 mg/kg ip	2-Deoxy-D-Glucose (2-DG) 500 mg/kg sc, 4.5, 2.5, 0.5 hr pre and 1.5, 3.5 hr post MDMA	Competitive inhibitor of glucose uptake and metabolism	Protection	Hypothermia occurred and manual warming blocked protection	(Hervias et al. 2000)
40 mg/kg sc	CGS-19755 25 or 50 mg/kg twice, 15 m pre, 40 m post MDMA	Competitive NMDA (AMPA) antagonist	Protection	Hypothermia	(Farfel and Seiden 1995)
20 mg/kg sc	alpha-methyl-para-tyrosine 100 mg/kg twice, 3 h pre and along withly MDMA	Competitive tyrosine hydroxylase inhibitor	Protection	NA	(Schmidt et al. 1990c); also (Schmidt and Kehne 1990)
20 mg/kg sc twice, 6 hr apart	ethanol 0.5 g/kg IP twice, 6 hr apart, along with MDMA	Complex	No protection	Unchanged	(Yeh 1999)
20 mg/kg sc twice, 6 hr apart	ethanol 1.0 g/kg IP twice, 6 hr apart, along with MDMA	Complex	No protection	Hypothermia	(Yeh 1999)
20 mg/kg sc twice, 6 hr apart	ethanol 2.0 g/kg IP twice, 6 hr apart, along with MDMA	Complex	No protection	Hypothermia	(Yeh 1999)
20 mg/kg ip	gamma-butyrolactone 400 mg/kg ip, 5 m pre and 55 m post MDMA	Complex, GHB analog/precursor	Some protection	NA	(Colado and Green 1994)

Table 4.2. Studies of Drugs Modifying MDMA Neurotoxicity and Hyperthermia

MDMA REGIMEN (dose, route)	OTHER INTERVENTION	CATEGORY OF OTHER INTERVENTION	OUTCOME OF MDMA + OTHER INTERVENTION	HYPER-THERMIA?	REFERENCE
10 mg/kg ip twice daily for 3 d	N,alpha-dimethylpiperonylamine 20 mg/kg ip twice daily for 3 d, along with MDMA	Compound with 3,4-methylenedioxyphenyl group	No protection	NA	(Hashimoto et al. 1992a)
15 mg/kg ip	haloperidol, 2 mg/kg ip, 5 m pre and 55 m post MDMA	DA antagonist	Some protection	Normthermia; protection lost in high ambient temperature	(Colado et al. 1999c)
30 mg/kg MDMA HCl sc	haloperidol, 2 mg/kg ip, 15 min pre MDMA	DA antagonist	Protection	Hypothermia	(Aguirre et al. 1998a)
20 mg/kg ip twice, 6 hr apart	haloperidol 2 mg/kg ip, 10 m pre each MDMA injection MDMA	DA antagonist	Some protection	Attenuated hyperthermia	(Hewitt and Green 1994)
7.5 mg/kg sc every 2 hr x 4	haloperidol 1 mg/kg ip every 2 hr x 4, 30 m pre each MDMA injection MDMA	DA antagonist	Some protection	NA	(Finnegan et al. 1993)
20 mg/kg sc	haloperidol 1 mg/kg sc, post 3 hr MDMA	DA antagonist	No protection	NA	(Schmidt et al. 1990a)
20 mg/kg sc	haloperidol 2 mg/kg ip, along with MDMA	DA antagonist	Some protection	Unchanged	(Schmidt et al. 1990b)
20 mg/kg sc	haloperidol 2 mg/kg ip, along with MDMA	DA antagonist	Protection	NA	(Schmidt et al. 1990c)
20 mg/kg sc	alpha-methyl-para-tyrosine 120 mg/kg ip, 90 m pre MDMA	DA depletor	Moderate protection	NA	(Stone et al. 1988)
20 mg/kg sc	unilateral 8-OHDA 12 ug in substantia niagra, 7 d pre MDMA	DA neurotoxin	No protection on both lesioned and unlesioned sides	NA	(Schmidt et al. 1990c)
20 mg/kg sc	bilateral 8-OHDA 12 ug in substantia niagra, 7 d pre MDMA	DA neurotoxin	Some protection	NA	(Schmidt et al. 1990c)

Table 4.2. Studies of Drugs Modifying MDMA Neurotoxicity and Hyperthermia

MDMA REGIMEN (dose, route)	OTHER INTERVENTION	CATEGORY OF OTHER INTERVENTION	OUTCOME OF MDMA + OTHER INTERVENTION	HYPER-THERMIA?	REFERENCE
15 mg/kg ip	L-Dopa 25 mg/kg and benserazide 6.25 mg/kg ip, 2 hr post MDMA	DA precursor and peripheral Dopa decarboxylase inhibitor	No protection or exacerbation	Prolonged hyperthermia	(Colado et al. 1999c)
20 mg/kg d-MDMA sc, four times, every 2 hrs	cocaine HCl 100 mg/kg sc, 20 m before 1st and 3rd injection MDMA	DA reuptake blocker	No protection	Attenuated hyperthermia	(Miller and O'Callaghan 1994)
10 mg/kg ip every 2 hrs x 4	mazindol 5 mg/kg ip repeated 4 times, 30 min pre MDMA	DA uptake inhibitor	Protection	Unchanged	(Shankaran et al. 1999b)
20 mg/kg sc	GBR 12909 20 mg/kg ip, 12 m pre MDMA	DA uptake inhibitor	Moderate protection	NA	(Stone et al. 1988)
15 mg/kg ip	N-tert-Butyl-alpha-Phenylnitrone 120 mg/kg route??? twice, 125 m apart, 10 m pre, 120 m post MDMA	Free radical scavenger	Some protection	Unchanged	(Colado et al. 1997b)
10 mg/kg ip	N-tert-Butyl-alpha-Phenylnitrone 150 mg/kg IP twice, 10 m pre, 120 m post MDMA	Free radical scavenger	Some protection	Attenuated hyperthermia	(Colado and Green 1995)
10 mg//kg sc, twice daily for 4 days	sodium salicylate (3.1 - 100 mg/kg ip, repeated), 1 hr pre MDMA	Free radical trapper, but also antipyretic	No protection	Hyperthermia	(Yeh 1997)
20 mg//kg sc	sodium salicylate (12.5 - 400 mg/kg ip), 1 hr pre MDMA	Free radical trapper, but also antipyretic	Exacerbated toxicity (5HT decrease)	Increased	(Yeh 1997)
20 mg/kg sc twice, 6 hr apart	N-tert-Butyl-alpha-Phenylnitrone (50, 100, 200, or 400mg/kg IP and ethanol 0.5 or 1.0 g/kg IP) twice, 6 hr apart, along with MDMA	Free radical trapping agent	Protection	Hypothermia	(Yeh 1997)
15 mg/kg ip	pentobarbitone 25 mg/kg ip twice, 5 min pre, 55 min post MDMA	GABA enhancer	No protection	"Brief" hypothermia	(Colado et al. 1999a)

Table 4.2. Studies of Drugs Modifying MDMA Neurotoxicity and Hyperthermia

MDMA REGIMEN (dose, route)	OTHER INTERVENTION	CATEGORY OF OTHER INTERVENTION	OUTCOME OF MDMA + OTHER INTERVENTION	HYPER-THERMIA?	REFERENCE
15 mg/kg ip	pentobarbitone 40 mg/kg ip twice, 5 min pre, 55 min post MDMA	GABA enhancer	Protection	"Sustained" hypothermia; protection abolised by induced hyperthermia	(Colado et al. 1999a)
20 mg/kg ip	pentobarbitone na (25 mg/kg ip), 5 m pre and 55 m post MDMA	GABA enhancer	Some protection	NA	(Colado and Green 1994)
20 mg/kg ip twice, 6 hr apart	chlormethiazole 100 mg/kg ip, 10 m pre each MDMA injection MDMA	GABA enhancer	Some protection	Normothermia	(Hewitt and Green 1994)
15 mg/kg ip	chlormethiazole 50 mg/kg ip, 5 m pre, 55 m post MDMA	GABA potentiator	Protection	Modest hypothermia; protection reduced by high ambient temperature	(Colado et al. 1998)
40 mg/kg sc	Dizocilpine (MK-801) 2.5 mg/kg and hot ambient temperature, 15 m pre MDMA	Glutamate receptor antagonist; Heat	No protection	Hyperthermia due to ambient temperature	(Farfel and Seiden 1995)
50 mg/kg ip	Human SOD expression, - MDMA	Increased endogenous antioxidant enzymes	-	-	(Cadet et al. 1995)
50 mg/kg ip, daily for 3 d	Human SOD expression, - MDMA	Increased endogenous antioxidant enzymes	-	-	(Cadet et al. 1995)
20 mg/kg sc	reserpine 5 mg/kg sc, 18 hr pre MDMA	Inhibits vesicular storage of monoamines	Some protection	NA	(Schmidt et al. 1990c); also (Schmidt and Kehne 1990)
30 mg/kg ip	reserpine 5 mg/kg ip, 24 hr pre MDMA	Inhibits vesicular storage of monoamines	No protection	NA	(Hekmatpanah et al. 1989)

Table 4.2. Studies of Drugs Modifying MDMA Neurotoxicity and Hyperthermia

MDMA REGIMEN (dose, route)	OTHER INTERVENTION	CATEGORY OF OTHER INTERVENTION	OUTCOME OF MDMA + OTHER INTERVENTION	HYPER-THERMIA?	REFERENCE
20 mg/kg sc	reserpine 5 mg/kg, 12 hr pre MDMA	Inhibits vesicular storage of monoamines	Protection	NA	(Stone et al. 1988)
20 mg/kg sc	monofluoromethyl DOPA 100 mg/kg IP daily for 3 d, 3, 2 and 1 d pre MDMA	Irreversible l-aromatic amino acid decarboxylase inhibitor	Some protection	NA	(Schmidt et al. 1990c); also (Schmidt and Kehne 1990)
15 mg/kg ip	AR-B15896AR 20 mg/kg ip twice, 5 m pre, 55 m post MDMA	Low affinity NMDA channel blocker	No protection	Unchanged	(Colado et al. 1998)
7.5 mg/kg sc every 2 hr x 4	flunarizine 15 mg/kg ip every 2 hr x 4, 30 m pre each MDMA injection MDMA	L-type calcium channel antagonist	Moderate Protection	NA	(Finnegan et al. 1993)
7.5 mg/kg sc every 2 hr x 4	flunarizine 20 mg/kg ip every 2 hr x 4, 30 m pre each MDMA injection MDMA	L-type calcium channel antagonist	Protection	NA	(Finnegan et al. 1993)
7.5 mg/kg sc every 2 hr x 4	haloperidol 1 mg/kg ip every 2 hr x 4, 30 m pre each MDMA injection MDMA	L-type calcium channel antagonist	Some protection	NA	(Finnegan et al. 1993)
7.5 mg/kg sc every 2 hr x 4	nifedipine 25 or 30 mg/kg ip three times, 30 m pre 1st, 2nd, and 4th MDMA injections MDMA	L-type calcium channel antagonist	No protection	NA	(Finnegan et al. 1993)
7.5 mg/kg sc every 2 hr x 4	verapamil 20 mg/kg ip three times, 30 m pre 1st, 2nd, and 4th MDMA injections MDMA	L-type calcium channel antagonist	No protection	NA	(Finnegan et al. 1993)
7.5 mg/kg sc every 2 hr x 4	verapamil 50 mg/kg ip three times, 30 m pre 1st, 2nd, and 4th MDMA injections MDMA	L-type calcium channel antagonist	No protection	NA	(Finnegan et al. 1993)
40 mg/kg sc	L-deprenyl (2 mg/kg IP), 30 m pre MDMA	MAO-B inhibitor	Some protection	NA	(Sprague and Nichols 1995a)

Table 4.2. Studies of Drugs Modifying MDMA Neurotoxicity and Hyperthermia

MDMA REGIMEN (dose, route)	OTHER INTERVENTION	CATEGORY OF OTHER INTERVENTION	OUTCOME OF MDMA + OTHER INTERVENTION	HYPER-THERMIA?	REFERENCE
40 mg/kg sc	MDL-72974 (1.25 mg/kg ip), 30 m pre MDMA	MAO-B inhibitor	Some protection	NA	(Sprague and Nichols 1995a)
20 mg/kg sc	desipramine 10 mg/kg ip, 60 m pre MDMA	NE reuptake inhibitor	Protection	NA	(Shankaran and Gudelsky 1998)
20 mg/kg ip	chlormethiazole 50 mg/kg ip twice, 5 m pre, 55 m post MDMA	Neuroprotective	Protection	NA	(Colado et al. 1993)
20 mg/kg ip	chlormethiazole 100 mg/kg ip, 20 m post MDMA	Neuroprotective	Some protection	Attenuated hyperthermia	(Colado et al. 1993)
40 mg/kg ip	Nw-nitro-L-arginine (10 mg/kg x 2), 30 m pre, 16 h post MDMA	Nitric oxide synthase inhibitor	Partial protection of 5HT in 2 of 6 regions	Unchanged	(Zheng and Laverty 1998)
10 mg//kg sc, every 2 hr x 4	L-NAME (150 mg/kg ip, twice), 30 min prior to 1st and 4th MDMA doses MDMA	Nitric oxide synthase inhibitor	Some protection	NA	(Taraska and Finnegan 1997)
10 mg//kg sc, every 2 hr x 4	NG-nitro-L-arginine (50 mg/kg ip, twice daily for 4 days), last injection was 14 hr pre MDMA MDMA	Nitric oxide synthase inhibitor	No protection	NA	(Taraska and Finnegan 1997)
10 mg/kg sc every 2 hrs x 4	Dizocilpine (MK-801) 2 mg/kg sc every 2 hrs x 4, along with MDMA	NMDA antagonist	Moderate Protection	NA	(Finnegan and Taraska 1996)
20 mg/kg sc, d-MDMA, every 2 hr x 4	Dizocilpine (MK-801) (1.0 mg/kg sc, twice), 30 min pre 1st and 3rd dose MDMA	NMDA antagonist	Protection	Hypothermia noted	(Miller and O'Callaghan 1995)
20 mg/kg sc, d-MDMA, every 2 hr x 4	Dizocilpine (MK-801) (1.0 mg/kg sc, twice) 30 min pre 1st and 3rd dose MDMA	NMDA antagonist	Protection	Normothermia	(Miller and O'Callaghan 1995)
20 mg/kg sc, d-MDMA, every 2 hr x 4	Dizocilpine (MK-801) (1.0 mg/kg sc, twice), 30 min pre 1st and 3rd dose MDMA	NMDA antagonist	Protection	Hypothermia noted	(Miller and O'Callaghan 1995)

Table 4.2. Studies of Drugs Modifying MDMA Neurotoxicity and Hyperthermia

MDMA REGIMEN (dose, route)	OTHER INTERVENTION	CATEGORY OF OTHER INTERVENTION	OUTCOME OF MDMA + OTHER INTERVENTION	HYPER-THERMIA?	REFERENCE
20 mg/kg ip twice, 6 hr apart	Dizocilpine (MK-801) 1 mg/kg, 10 m pre each MDMA injection MDMA	NMDA antagonist	Some protection	Attenuated hyperthermia	(Hewitt and Green 1994)
20 mg/kg d-MDMA sc, four times, every 2 hrs	Dizocilpine (MK0801) 1.0 mg/kg sc, 20 m before 1st and 3rd injection MDMA	NMDA antagonist	Some protection	Hypothermia occurred and high ambient temperature blocked protection	(Miller and O'Callaghan 1994)
20 mg/kg d-MDMA sc, four times, every 2 hrs	Dizocilpine (MK-801) (1.0 mg/kg sc, twice), 20 m before 1st and 3rd injection MDMA	NMDA antagonist	Protection	NA	(O'Callaghan and Miller 1994)
20 mg/kg ip	Dizocilpine (MK-801) 1 mg/kg ip twice, 5 m pre, 55 m post MDMA	NMDA antagonist	Some protection	NA	(Colado et al. 1993)
25 mg/kg d-MDMA sc, every 2 hr x 4	Dizocilpine (MK-801) 1.0 mg/kg sc, 30 m pre 1st and 3rd MDMA injections MDMA	NMDA antagonist	NA	NA	(Miller and O'Callaghan 1993)
20 mg/kg sc	Dizocilpine (MK-801) 2.5 mg/kg, 15 m pre MDMA	NMDA antagonist	Some protection	NA	(Farfel et al. 1992)
40 mg/kg sc	Dizocilpine (MK-801) 0.5 mg/kg, 15 m pre MDMA	NMDA antagonist	No protection	NA	(Farfel et al. 1992)
40 mg/kg sc	Dizocilpine (MK-801) 1.0 mg/kg, 15 m pre MDMA	NMDA antagonist	No protection	NA	(Farfel et al. 1992)
40 mg/kg sc	Dizocilpine (MK-801) 2.5 mg/kg, 15 m pre MDMA	NMDA antagonist	Some protection	NA	(Farfel et al. 1992)
10 mg/kg sc five times, every 6 hr	dextrorphan 7.5-45.0 m/kg five times, every 6 hr, 20 m pre each injection MDMA	NMDA antagonist	Protection (dose-dependent)	NA	(Finnegan et al. 1990)

Table 4.2. Studies of Drugs Modifying MDMA Neurotoxicity and Hyperthermia

MDMA REGIMEN (dose, route)	OTHER INTERVENTION	CATEGORY OF OTHER INTERVENTION	OUTCOME OF MDMA + OTHER INTERVENTION	HYPER-THERMIA?	REFERENCE
100 mg/kg twice daily for 2 d	Dizocilpine (MK-801) 1.0 mg/kg , 30 m pre each dose MDMA	NMDA antagonist	Protection ("virtually eliminated silver staining")	NA	(Jensen et al. 1993)
20 mg/kg d-MDMA sc, four times, every 2 hrs	diethyldithiocarbamate HCl 400 mg/kg sc, 20 m before 1st and 3rd injection MDMA	NO trapping agent, SOD inhibitor, metal chelator	Some protection	Hypothermia	(Miller and O'Callaghan 1995)
40 mg/kg sc	NBQX 55 mg/kg three injections 15 m apart, 15 m pre, along with, 15 m post MDMA	Noncompetitive NMDA antagonist	No protection	Hyperthermia or Normothermia	(Farfel and Seiden 1995)
15 mg/kg ip	Nicotinamide 200 mg/kg ip, 0.5 hr pre, along with, 2, 4 hr post MDMA	Precursor for the electron carrier NAD, potentially improving mitochondrial energy production but also possibly altering MDMA metabolism	Exacerbated	Hyperthermia	(Hervias et al. 2000)
20 mg/kg d-MDMA sc, four times, every 2 hrs	pentobarbital 50 mg/kg sc, 20 m before 1st and 3rd injection MDMA	Sedative-Hypnotic	Some protection	Hypothermia	(Miller and O'Callaghan 1994)
20 mg/kg sc	fluoxetine, 10 mg/kg ip, 1 hr pre MDMA	SSRI	Protection	Unchanged	(Shankaran et al. 1999a)
20 mg/kg sc	fluoxetine, 10 mg/kg ip, 4 hr post MDMA	SSRI	Protection	Unchanged	(Shankaran et al. 1999a)
30 mg/kg MDMA HCl sc	fluoxetine, 5 mg/kg sc, 15 min pre MDMA	SSRI	Protection	Hyperthermia	(Aguirre et al. 1998a)
10 mg/kg ip twice daily for 3 d	paroxetine 10 mg/kg ip twice daily for 3 d, along with MDMA	SSRI	Some protection	NA	(Hashimoto et al. 1992a)
10 mg/kg ip twice daily for 3 d	6-nitroquipazine 10 mg/kg ip twice daily for 3 d, along with MDMA	SSRI	Protection	NA	(Hashimoto et al. 1992a)

Table 4.2. Studies of Drugs Modifying MDMA Neurotoxicity and Hyperthermia

MDMA REGIMEN (dose, route)	OTHER INTERVENTION	CATEGORY OF OTHER INTERVENTION	OUTCOME OF MDMA + OTHER INTERVENTION	HYPER-THERMIA?	REFERENCE
20 mg/kg sc	MDL 27,777 5 mg/kg sc, post 3 hr MDMA	SSRI	Some protection	NA	(Schmidt et al. 1990a)
10 mg/kg sc	MDL 27,777 5 mg/kg sc, along with MDMA	SSRI	"Full" protection	Attenuated hyperthermia	(Schmidt et al. 1990b)
20 mg/kg sc	fluoxetine 5 mg/kg sc, along with MDMA	SSRI	Protection	NA	(Schmidt 1987)
20 mg/kg sc	fluoxetine 5 mg/kg sc, post 3 hr MDMA	SSRI	Protection	NA	(Schmidt 1987)
20 mg/kg sc	fluoxetine 5 mg/kg sc, post 6 hr MDMA	SSRI	Possible protection	NA	(Schmidt 1987)
20 mg/kg sc	fluoxetine 5 mg/kg sc, post 12 hr MDMA	SSRI	No protection	NA	(Schmidt 1987)
100 mg/kg twice daily for 2 d	fluoxetine 5 mg/kg twice daily for 2 d, 30 m pre each dose MDMA	SSRI	Protection (decreased vol and intensity of silver stained tissue in frontalparietal cortex)	NA	(Jensen et al. 1993)
10 mg/kg ip twice daily for 3 d	6-nitroquipazine 5 mg/kg ip, along with MDMA	SSRI	Protection	NA	(Hashimoto and Goromaru 1990)
20 mg/kg sc, d-MDMA, every 2 hr x 4	restraint, from 30 min pre to 30 min post MDMA	Stressor	Some protection	Hypothermia noted	(Miller and O'Callaghan 1995)
20 mg/kg sc	adrenalectomy, before MDMA	Surgical removal of glucocorticoid source	Some protection	NA	(Johnson et al. 1989)
20 mg/kg ip	p-chlorophenylalanine (PCPA) 150 mg/kg ip daily for 3 d, daily for 3 d, ending 1 d pre MDMA	Tryptophan hydroxylase inhibitor	No protection	NA	(Brodkin et al. 1993)

Table 4.2. Studies of Drugs Modifying MDMA Neurotoxicity and Hyperthermia

MDMA REGIMEN (dose, route)	OTHER INTERVENTION	CATEGORY OF OTHER INTERVENTION	OUTCOME OF MDMA + OTHER INTERVENTION	HYPER-THERMIA?	REFERENCE
20 mg/kg sc	adrenalectomy AND corticosterone 10 mg sc, 24 hr and 15 m pre, 24 h post MDMA	Surgical removal of glucocorticoid source and injection of corticosterone	Exacerbated toxicity compared to adrenalectomy alone	NA	(Johnson et al. 1989)
40 mg/kg sc	alpha-methyl-para-tyrosine (75 mg/kg ip, twice), 5 and 1 hr pre MDMA	Tyrosine hydroxylase inhibitor (DA depleter)	Protection	Hypothermia occurred and manual warming blocked protection	(Malberg et al. 1996)
20 mg/kg ip	alpha-methyl-para-tyrosine, 250 mg/kg ip, 2 hr pre MDMA	Tyrosine hydroxylase inhibitor (DA depleter)	Protection	NA	(Brodkin et al. 1993)

The following papers have not been obtained and are therefore not included in this table: (Gibb et al. 1997; Murray et al. 1996; Vorhees 1997)

dopaminergic neurotoxicity (Melega et al. 1998). In contrast, hypothermia protects against methamphetamine-induced dopaminergic neurotoxicity in rodents (Ali et al. 1994; Miller and O'Callaghan 1994). Thus, the influence of temperature on phenethylamine neurotoxicity remains to be demonstrated in primates.

Drugs Modifying MDMA Neurotoxicity

Many studies have coadministered another drug in order to investigate the neurochemical mechanisms that produce MDMA neurotoxicity. However, the recognition that animal core temperature influences MDMA neurotoxicity has forced researchers to re-evaluate many of these studies. Drug interaction studies investigating MDMA neurotoxicity are summarized in **Table 4.2**. As can be seen, the role of temperature in many of these studies is still unclear.

Time Course of Changes and Extent of Recovery

High doses of MDMA have a biphasic effect on indices of serotonergic functioning, causing acute decreases, then partial recovery, and then chronic decreases in these indices. For example, after a single dose of 10 mg/kg MDMA to a rat, release of 5HT leads to depletion of tissue levels of 5HT and its metabolite 5HIAA within 3 hrs of dosing (Schmidt 1987; Stone et al. 1987b). At approximately post 6 hrs, levels begin to return to normal, but this recovery is not sustained. About 24 hr after dosing, 5HT levels begin a second, sustained decrease and remain significantly lower than baseline 2 weeks later. This sustained decrease is thought to be associated with axonal degeneration.

The intracellular enzyme TPH follows a similar time course, with decreased activity occurring within 15 min of drug administration. However, there is less short-term recovery of TPH activity in comparison to 5HT. This recovery of TPH activity appears to involve regeneration of oxidatively inactivated enzyme rather than synthesis of new enzyme. SERT functioning is also altered. Uptake of radiolabelled 5HT is decreased to 80% of control levels in tissue prepared from striata of rats sacrificed at one hour after 15 mg/kg subcutaneous MDMA (Fleckenstein et al. 1999). It should be noted that significant acute 5HT depletions are not necessarily produced by all active doses of MDMA. In Sprague Dawley rats, 2.5 mg/kg MDMA did not produce an acute decrease in 5HT or 5HIAA at 3 hours after injection (Schmidt et al. 1986). Striatal 5HT depletions were found in a chronic MDMA user who died shortly after MDMA ingestion (Kish et al. 2000). This suggests that at least some of the doses administered by illicit users are sufficient to produce 5HT depletions.

The above description focuses on serotonergic changes because these are used to measure toxicity. Many other acute neurochemical changes occur after MDMA exposure. For example, dopamine is released (Stone et al. 1986) and dopamine transporter reuptake activity is decreased within 1 hr of high dose MDMA (Fleckenstein et al. 1999; Metzger et al. 1998). MDMA can also acutely increase dopamine synthesis (Nash 1990). Mice are selectively vulnerable to MDMA-induced dopaminergic neurotoxicity (Logan et al. 1988; Miller and O'Callaghan 1994; Stone et al. 1987a). In some studies, long-term alterations

in dopaminergic functioning have been seen in other species, such as rats (Commins et al. 1987b).

The time course of damaging events in rats can be seen by administering SSRIs, such as fluoxetine and citalopram, after MDMA. Administering fluoxetine or citalopram before or simultaneous to MDMA has been shown to block the neurotoxicity of MDMA (Battaglia et al. 1988a; Malberg et al. 1996; Schmidt 1987; Schmidt and Taylor 1990; Shankaran et al. 1999a; Virden and Baker 1999), probably by blocking interactions of MDMA with SERT (Berger et al. 1992b). More interestingly, fluoxetine remains almost fully protective if given 3 or 4 hours after MDMA. By 4 hrs, most of the MDMA-induced release of 5HT and DA has already occurred (Gough et al. 1991; Hiramatsu and Cho 1990) and increases in extracellular free radicals (Colado et al. 1997b; Shankaran et al. 1999a) and lipid peroxidation (Colado et al. 1997b) can be measured. Nevertheless, the administration of fluoxetine at this point decreases subsequent extracellular oxidative stress (Shankaran et al. 1999a) and long-term 5HT depletions (Schmidt 1987; Shankaran et al. 1999a). Fluoxetine will still be partially protective if given 6 hrs after MDMA but has no protective effect at post 12 hrs (Schmidt 1987). This shows that neurotoxic MDMA regimens initiate a series of events that become increasingly damaging between 3 and 12 hrs after drug administration in rats.

Slow recovery of indices of serotonergic functioning can be seen following a neurotoxic dose of MDMA. Recovery is probably at least partially due to axonal regrowth since increases follow a regionally specific pattern. For example, there is a rostral to caudal pattern of recovery in the cortex and hippocampus (Lew et al. 1996; Molliver et al. 1990). It appears that recovery is greatest in brain regions that are nearer to 5HT nerve cell bodies in the raphe or major 5HT axon bundles (Fischer et al. 1995; Hatzidimitriou et al. 1999). Interestingly, serotonergic indices have been found to continue to increase beyond their normal levels during recovery in some nearer areas such as the amygdala and hypothalamus (Fischer et al. 1995; Lew et al. 1996; Ricaurte et al. 1992; Sabol et al. 1996). Thus, “recovery” does not necessarily mean that the animal returns to its predrug state.

Extent of recovery is different in different species. In rats, there is extensive recovery of indices of serotonergic functioning 1 year after drug exposure (Battaglia et al. 1988b; Lew et al. 1996; Sabol et al. 1996; Scanzello et al. 1993), although there is significant variation in recovery between individual animals (Fischer et al. 1995). In primates, some recovery of serotonergic function occurs but is less extensive than in the rat. Altered serotonergic axon density was still detectable 7 years after MDMA exposure in one study employing squirrel monkeys (Hatzidimitriou et al. 1999). Therefore, despite some recovery, MDMA-induced serotonergic changes are likely permanent in this primate species. This apparent species difference may be partially related to the more severe initial serotonergic damage usually seen in primates compared to rats, but likely also indicates a species difference in regrowth of serotonergic axons.

MDMA-Induced Apoptosis (Programmed Cell Death)

While MDMA has generally been found to selectively affect axons rather than cell bodies, two *in vitro* studies have suggested that MDMA may trigger programmed cell death under certain conditions. In one study, exposure to MDMA for 48 hrs dose-dependently decreased survival of cultured human placental serotonergic cells (Simantov and Tauber 1997). This decreased cell viability was accompanied by DNA fragmentation and cell cycle arrest (in the G2M phase). 48 hr exposure to 0.4 mM MDMA decreased cell survival by 1.4 ± 4%, while 1.2 mM MDMA decreased cell survival by 61 ± 9%. This cytotoxicity appeared to be the result of a specific pharmacological interaction as cultured dopaminergic cells were unaffected and toxicity to serotonergic cells was dose-dependently blocked by imipramine. In another study, the effects of MDMA and structurally related amphetamines on cultured rat neocortical neurons were studied at concentrations of 125 to 1000 μM MDMA and exposure times of 1, 24, and 96 hours (Stumm et al. 1999). Cell survival was not significantly affected by 125 μM MDMA at any exposure time. However, cell survival was decreased by 34.2 ± 11.4% at 96 hours after an average exposure of 500 μM MDMA. Stumm et al. also noted DNA fragmentation and altered expression of the bcl-x_{LS} gene, which supports the interpretation that programmed cell death had occurred. The degree of cytotoxicity noted for MDMA in this study was comparable to the toxicity produced by other structurally related amphetamines.

The relevance of these *in vitro* studies to humans is difficult to assess because MDMA concentrations likely differ in the brain and blood. In a human fatality, MDMA concentrations were 4.8 times higher in brain than blood (Rohrig and Prouty 1992). However, postmortem changes in drug levels are possible. If it is assumed that postmortem redistribution did not occur and MDMA levels in the brain are about 5 times higher than in blood, then 150 mg MDMA might produce peak brain levels of 2.5 mg/L. This estimated peak level is significantly less than the lowest drug concentration used in either study (0.4 μM MDMA is 77.3 mg/L, while 125 μM is 24.2 mg/L). Given these concentration differences and the long exposure times used in these studies, it does not seem likely that human oral doses of MDMA would be sufficient to induce programmed cell death.

Non-serotonergic MDMA Neurotoxicity in the Somatosensory Cortex

Non-serotonergic cell damage has been detected after MDMA exposure to the rat somatosensory cortex using silver staining. This region is similarly affected by methamphetamine and amphetamine (Commins et al. 1987b; Ryan et al. 1990). Research with methamphetamine has shown that these cells are likely glutamatergic (Pu et al. 1996) and may comprise a corticostriatal pathway. MDMA-induced silver staining in this region was detected after 80 mg/kg MDMA exposure in Sprague-Dawley rats (Commins et al. 1987a).

Behavioral and Functional Correlates of MDMA Exposure in Animals

A number of studies have looked for evidence that MDMA neurotoxicity causes lasting behavioral or functional changes in nonhuman animals. These studies are summarized in **Table 4.3** and are, perhaps, impressive for the limited nature of their behavioral findings. It is clear that neurotoxic MDMA exposure can both alter neurochemical functioning and the response of animals to subsequent drug exposures. However, so far five published studies suggest that drug-free MDMA-exposed animals also have behavioral alterations or functional impairments at seven or more days after last MDMA exposure.

Lasting thermoregulatory impairment has been demonstrated in MDMA-exposed animals by two research groups (Dafters and Lynch 1998; Mechan et al. 2001). In the earlier study, rats were placed in a warm environment (30° C) fourteen weeks after exposure to a neurotoxic MDMA or placebo regimen. MDMA-exposed rats had significantly larger increases in core temperature than control rats. In the second study, a similar effect was seen 5 to 6 wk after neurotoxic MDMA exposure (Mechan et al. 2001). Rats that had been previously exposed to MDMA had a quicker increase in core temperature when placed in a warm environment (30° C) and had a longer-lasting increase in temperature when removed from that environment. It has been known for many years that individuals who experience heat stroke have increased susceptibility to subsequent episodes for some time (Shapiro et al. 1979) and it appears possible that the same phenomenon is being detected here. Alternatively, this may be an effect of neurotoxic changes in the hypothalmus. The serotonergic neurotoxin 5,6-dihydroxytryptamine (5,6-DHT) produces a similar thermoregulatory impairment when injected into the rat anterior hypothalmus (Myers 1975).

Rats exposed to a neurotoxic MDMA regimen showed reductions in diurnal and nocturnal locomotor activity at 7 to 14 days after drug treatment (Wallace et al. 2001). Neostriatal 5HT levels were measured at 15 to 17 days after treatment and were found to be 31% of control levels. It is not clear if this reduction in activity is the result of neurotoxicity rather than some other non-neurotoxic effect of MDMA. Some regimens of repeated non-neurotoxic exposure to d-amphetamine can reduce nocturnal activity in rats (Robinson and Camp 1987). The authors speculate that the hyperactivity in MDMA-treated rats may relate to a possible dysregulation of the sleep-wake cycle, which is thought to be influenced by serotonergic neurons originating in the dorsal raphe nucleus. This hypothesis is consistent with the reported differences in sleep between MDMA users and nonusers (Allen et al. 1993; McCann et al. 2000).

Two studies have suggested that neurotoxic MDMA exposure may cause neurocognitive impairment in rats. The first study used adult animals and the second study used newborn rats. In the study with adult animals, drug-free alterations in performance were detected using a delayed memory task (Marston et al. 1999). MDMA-exposed rats displayed significantly reduced accuracy and increased response bias at the longer (20 or more sec) delays between presentation of information and testing. This difference appeared to be the result of less improvement in accuracy by the MDMA-exposed

Table 4.3. Studies of Long-term Behavioral or Functional Changes after MDMA Exposure in Animals

Species and Strain	Neurotoxic MDMA Regimen	Significant Effects of MDMA Exposure	Measures not Significantly Different	Reference
Rhesus Monkeys	10 mg/kg IM, twice a day, for 4 days	Right shift in MDMA and d-fenfluramine dose-response curve for time estimation, learning task, and motivation tasks at post 1 mo.	Drug-free performance on all tasks at post 1 mo.	(Frederick et al. 1998)
Rhesus Monkeys	Escalating doses of 0.10, 0.3, 1.0, 1.75, 3.0, 5.6, 7.5, 10.0, 15.0, and 20 mg/kg, IM, twice daily for 14 consecutive days at each dose. (2 of 3 animals 'skipped' the 1.75, 7.5, and 15 mg/kg dose levels). Preceded by one period of dose response testing using doses up to 1.75 mg/kg IM and followed by three two month periods of dose-response testing using doses up to 5.7 mg/kg IM.	Right shift in MDMA dose-response curve for time estimation, short-term memory, color and position discrimination, and motivation tasks post 5, 12 or 19 mo from chronic regimen (post 12 and 19 mo measures were post 5 mo from a dose-response determination).	Drug-free performance on all tasks at all time points.	(Frederick et al. 1995)
Rhesus Monkeys	10 mg/kg IM, twice a day for 4 days	Altered latencies of brainstem auditory evoked EEG potentials (P3 faster at post 2, 9, and 13 wk; P4 faster at post 2, 4, 9, and 13 wk; P5 slower at post 2 wk).	Tests of memory, reinforcer efficacy, bimanual coordination, and reaction time at all time points after post 2 wk; latencies of auditory and visual evoked EEG potentials at all time points; latencies of brainstem auditory evoked EEG potentials (P3 at 4, 17, and 21 wk; P4 at 17 and 21 wk; P5 at 4, 9, 13, 17, and 21 wk).	(Taffe et al. 2001)
Sprague-Dawley Rats	20 mg/kg SC, twice a day for 4 days	None, although researchers note that 2 of 8 MDMA-exposed rats failed to acquire lever pressing with 20 sec reinforcement delays during the 8 hr session at post 14 days.	Acquisition of and behavior on a lever-press responding task at post 14 days.	(Byrne et al. 2000)

Table 4.3 (continued). Studies of Long-term Behavioral or Functional Changes after MDMA Exposure in Animals

Species and Strain	Neurotoxic MDMA Regimen	Significant Effects of MDMA Exposure	Measures not Significantly Different	Reference
Sprague-Dawley Rats	10 mg/kg SC, twice a day for 4 days	Significant pretreatment x treatment x crossing times interaction, suggesting altered S(+)-MDMA -induced behavioral activation at post 21 days.	Drug-free locomotion at 21 days; RU24969-induced behavioral activation at 21 days.	(Callaway and Geyer 1992)
Wistar Rats	10 mg/kg SC, once per day for 4 days	Increased core temperature when placed in either 22 °C or 28 °C ambient temperature at post 4 or 14 wks.	None	(Dafters and Lynch 1998)
Long-Evans Rats	40 mg/kg SC, twice a day for 4 days	None	Sexual behaviors at post 10 days; Spontaneous motor activity.	(Dornan et al. 1991)
Sprague-Dawley Rats	20 mg/kg SC, twice a day for 4 days	Decreased cortical 5HT release in response to electrical stimulation in DRN at post 10-12 days.	Electrical-stimulated 5HT release in MRN or hippocampus at post 2 wks; Number and firing pattern of classical 5HT neurons and burst-firing neurons in DRN.	(Gartside et al. 1996)
Sprague-Dawley Rats	20 mg/kg SC, twice a day for 4 days	None	DOI-induced head twitch responses, locomotion, and rearing activity at post 1 mo.	(Granoff and Ashby 1998)
Sprague-Dawley Rats	20 mg/kg SC, twice a day for 4 days	Increased conditioned place preference response to cocaine in MDMA group at 2 post wks.	None	(Horan et al. 2000)
Sprague-Dawley Rats	5 mg/kg SC, once a day for 4 days, or 20 mg/kg SC, twice a day for 4 days, followed by 5 mg/kg MDMA 2 days later	Increased motor stimulant effects of 5.0 mg/kg SC MDMA or 15.0 mg/kg IP cocaine in both MDMA-treated groups at post 11 days; increased MDMA-stimulated DA release in the nucleus accumbens at post 2 wks.	Basal DA in nucleus accumbens at post 2 wks.	(Kalivas et al. 1998)

Table 4.3 (continued). Studies of Long-term Behavioral or Functional Changes after MDMA Exposure in Animals

Species and Strain	Neurotoxic MDMA Regimen	Significant Effects of MDMA Exposure	Measures not Significantly Different	Reference
Sprague-Dawley Rats	15 mg/kg IP	Loss of rate-dependence of response of nigrostriatal cells to either quinpirole or apomorphine at post 1 wk.	Basal activity of nigrostriatal DA neurons; Quinpirole-induced inhibition of nigrostriatal DA cell firing for all cells at post 1 wk.	(Kelland et al. 1989)
Sprague-Dawley Rats	6 mg/kg SC, twice a day daily for 4 days	Left shift in MDMA dose-response curve on DRL task in MDMA group at post 4 wks.	None	(Li et al. 1989)
Lister Hooded Rats	ascending regimen of 10, 15, and 20 mg/kg IP, each dose given twice daily for one day	Decreased performance in operant delayed match to nonsample task beginning at post 12 days.	Spontaneous behavior, body temperature, and skilled paw reach ("staircase task") up to post 16 days.	(Marston et al. 1999)
Dark Agouti Rats	12.5 mg/kg IP	Faster increase in core temperature in warm (30° C) environment and longer-lasting hyperthermia when returned to normal environment at post 33 days.	Hypothermic response to the $5HT_{1A}$ agonist 8-OH-DPAT	(Mechan et al. 2001)
Sprague-Dawley Rats	20 mg/kg SC, twice a day for 4 days	Increased cocaine-induced dopamine release in nucleus accumbens in MDMA group at post 2 wks.	None	(Morgan et al. 1997)
Sprague-Dawley Rats	20 mg/kg SC, twice a day for 4 days	Increased morphine-induced antinociception (assessed by tail flick test) at post 2 wks.	Drug free behavior in tail flick test at post 2 wks.	(Nencini et al. 1988)
Sprague-Dawley Rats	20 mg/kg SC, twice a day for 4 days	Decreased inhibitory effects of DA and SKF38393 on glutamate-evoked firing in nucleus accumbens cells at post 9-15 days.	Inhibitory effects of GABA on glutamate-evoked firing in nucleus accumbens cells at post 9-15 days.	(Obradovic et al. 1998)

Table 4.3 (continued). Studies of Long-term Behavioral or Functional Changes after MDMA Exposure in Animals

Species and Strain	Neurotoxic MDMA Regimen	Significant Effects of MDMA Exposure	Measures not Significantly Different	Reference
Sprague-Dawley Rats	20 mg/kg SC	Increased 8-OH-DPAT-induced prolactin release at post 2 wks. Decreased 8-OH-DPAT-stimulated ACTH release at post 2 wks.	Basal ACTH and prolactin concentrations and ACTH and prolactin response to saline injection at post 2 wks.	(Poland 1990)
Sprague-Dawley Rats	20 mg/kg SC	Increased d,l-Fenfluramine-stimulated prolactin release at post 2 wks and 4 mo. Decreased d,l-Fenfluramine-stimulated ACTH release at post 2 wks, 4 mo, and 8 mo.	d,l-Fenfluramine-stimulated ACTH at post 12 mo; d,l-Fenfluramine-stimulated prolactin at post 8 and 12 mo.	(Poland et al. 1997)
Sprague-Dawley Rats	20 mg/kg SC, twice a day for 4 days	Increased d,l-Fenfluramine-stimulated prolactin release at post 4 and 8 mo. Decreased d,l-Fenfluramine-stimulated ACTH release at post 4, 8, and 12 mo.	Saline-stimulated ACTH and prolactin release at post 2 weeks; d,l-Fenfluramine-stimulated prolactin release at post 12 mo.	(Poland et al. 1997)
Long-Evans Rats	20 mg/kg SC, twice a day for 4 days with entire regimen repeated 1 wk later	None	Performance in a spatial memory task using a T-maze (at post 7-10 wks) and scopolamine-induced changes in performance on this task (at post 15-16 wks).	(Ricaurte et al. 1993)
Sprague-Dawley Rats	10 mg/kg IP, twice a day for 4 days	None (increased time to find hidden platform in first trial of spatial navigation task at post 2 days).	Spatial navigation and learning set task at post 7-9 days, skilled reaching task at post 17-19 days, foraging task at post 26-29 days, with or without atropine pretreatment.	(Robinson et al. 1993)

Table 4.3 (continued): Studies of Long-term Behavioral or Functional Changes after MDMA Exposure in Animals

Species and Strain	Neurotoxic MDMA Regimen	Significant Effects of MDMA Exposure	Measures not Significantly Different	Reference
Sprague-Dawley Rats	20 mg/kg SC, twice a day for 4 days	Decreased discrimination of 1.0 mg/kg MDMA from saline at post 13-15 days.	Discrimination of 0.5 or 1.5 mg/kg; conditioned place preference from MDMA at post 13-15 days.	(Schechter 1991)
Sprague-Dawley Rats	10, 20, and 40 mg/kg SC, twice a day for 4 days	None	Food and water intake, schedule-controlled behavior, open field behavior, acquisition of one- and two- way avoidance, swimming ability, acquisition and extinction in 8-arm radial maze test, and morphine-induced antinociception at post 14-28 days.	Seiden, et al., 1993
Sprague-Dawley Rats	20 or 40 mg/kg IP, twice a day for 4 days	Decreased d-fenfluramine-stimulated 5HT release in frontal cortex at post 2 wks.	None	(Series et al. 1994)
Sprague-Dawley Rats	10 mg/kg IP, every 2 h for 4 injections	Decreased behavioral, hyperthermic, and 5HT-releasing effects of MDMA at 1 wk after neurotoxic regimen.	None	(Shankaran and Gudelsky 1999)
Sprague-Dawley Rats	20 mg/kg SC, twice a day for 4 days	Increased cerebral glucose utilization in molecular layer of dentate gyrus and in CA2 and CA3 fields of Ammon's horn in hippocampus at post 14 days.	Cerebral glucose utilization in neocortex, raphe nuclei, and some hippocampal areas at post 14 days.	(Sharkey et al. 1991)

Table 4.3 (continued): Studies of Long-term Behavioral or Functional Changes after MDMA Exposure in Animals

Species and Strain	Neurotoxic MDMA Regimen	Significant Effects of MDMA Exposure	Measures not Significantly Different	Reference
Sprague-Dawley Rats	5 or 10 mg/kg PO, daily for 4 days	None	Auditory startle, emergence from darkened chamber, complex maze navigation, response to hot plate, FI 90 operant behavioral task at post 2 to 4 weeks.	(Slikker et al. 1989)
Sprague-Dawley Rats	20 mg/kg, SC, twice a day for 4 days	Decreased S(+)-MDMA-appropriate responding after S(+)-MDMA and increased S(+)-MDMA-appropriate responding after saline at post 10 days.	None	(Virden and Baker 1999)
Sprague-Dawley Rats	10 mg/kg IP, every 2 hr for 4 injections	Decreased diurnal and nocturnal spontaneous locomotor activity at post 7 to 14 days.	Number of crossings into different quadrants of cage at all times.	(Wallace et al. 2001)
Young Sprague-Dawley Rat Pups	10 mg/kg SC, every 12 hrs for 4 or 7 injections	Decreased rate of ultrasonic vocalization measured up to post 11 days.	Behavioral responses to the $5HT_{1A}$ agonist 8-OH-DPAT, the $5HT_{1B}$ agonist TFMPP, and the $5HT_2$ agonist DOI at post 8 days.	(Winslow and Insel 1990)

Time from MDMA treatment is expressed as time from last exposure.
Abbreviations: DA - dopamine; DRN - dorsal raphe nucleus; DRL - differential reinforcement of low rate (behavioral task); FI - fixed interval of reinforcement (behavioral task); IM - intramuscular injection.

animals across days of testing and became statistically significant in post hoc comparisons beginning 12 days after last MDMA exposure. In contrast, other studies were unable to demonstrate any long-term effect of MDMA neurotoxicity on spatial navigation memory tasks in rats (Ricaurte et al. 1993; Robinson et al. 1993). However, Robinson and colleagues did detect short-term residual effects of MDMA on this task when animals were tested 2 days after the last MDMA exposure.

A recent study of newborn rats found that repeated MDMA exposure dose-dependently impaired sequential learning and spatial learning and memory (Broening et al. 2001). MDMA was administered twice daily for ten days, with individual doses of 5 to 20 mg/kg. Because of interspecies differences, newborn rats are thought to be the developmental equivalent of a third-trimester human fetus. Thus, the newborn rats were exposed to MDMA during the equivalent of the third-trimester in humans. Some rats were administered MDMA during the equivalent of the early part of the third trimester, while others were exposed during the late part of this period. MDMA decreased body

weight for both groups. In behavioral tests beginning at least 1 mo after MDMA exposure, rats exposed in the late (but not early) third trimester had significantly impaired learning and memory. These impairments were not correlated to the serotonergic neurotoxicity seen in both groups, which was measured as a 20% or less depletion of hippocampal serotonin.

Behavioral Effects of Other Serotonergic Neurotoxins

Given the apparently subtle behavioral effects of MDMA neurotoxicity, it may be useful to briefly survey the behavioral effects of other serotonergic neurotoxins such as 5,6 and 5,7 -DHT (hereafter "DHT neurotoxins"). As discussed earlier, neurotoxic MDMA exposure produces fewer consistent changes in markers of neural cell injury than DHT neurotoxins. This has been interpreted by some as evidence that MDMA is not truly neurotoxic. If MDMA also differed from DHT neurotoxins in behavioral effects, the difference could be seen as further evidence that MDMA is not neurotoxic. However, once the regional selectivity of these neurotoxins is taken into account, there are few clear differences in the behavioral and axonal effects of MDMA and other serotonergic neurotoxins.

For the purposes of this discussion, it is not necessary to distinguish between 5,6-DHT and 5,7-DHT. 5,7-DHT has greater chemical stability and typically causes fewer fatalities than 5,6-DHT. In both cases, researchers generally pretreat animals with the norepinephrine reuptake inhibitor desipramine to prevent damage to noradrenergic axons. Because of their polarity, DHT neurotoxins do not cross the blood-brain barrier and must be injected into a specific brain region or infused into the cerebral ventricles (allowing the toxin to spread throughout the brain). Thus, regionally selective or relatively global serotonergic neurotoxicity can be produced. MDMA is similar to DHT neurotoxins in that both damage serotonergic axons that subsequently regrew from surviving cell bodies and axons (Bjorklund and Stenevi 1979). MDMA differs from DHT neurotoxins in that MDMA is generally thought to only damage those serotonergic axons originating in the dorsal raphe nucleus. However, microinjection of DHT neurotoxins into the dorsal raphe nucleus should produce localized serotonergic changes similar to those seen in MDMA-induced neurotoxicity. Although the technique is less selective and may damage non-serotonergic cells and surrounding tissue, it is also possible to electrolytically lesion the dorsal raphe nucleus.

The effects of selective lesioning of the dorsal raphe serotonergic system are fairly subtle. An increase in motor activation was seen when DHT-lesioned rats were placed in a novel environment at 7 to 12 days after lesioning (Morrow and Roth 1996). This effect was transient and was not detectable after 12 days. Locomotor activity was correlated with 5HT levels in the median prefrontal cortex, which were an average of 35% of control levels. A study using electrolytical lesioning of the dorsal raphe nucleus reported decreased wheel running activity from 1 to 4, but not 5 to 8, weeks after lesioning in rats with forebrain SERT activity reduced to 54% of controls (Heym and Gladfelter 1982). Food and water intake and body weight were unaltered in the electrolytically-lesioned animals. The apparent discrepancy between these two studies may be due to the different

lesioning techniques or the environment in which behavior was measured. It may be that dorsal raphe lesioning transiently increases activity in novel environments while decreasing activity in familiar ones. Similarly transient changes were reported in a study that employed a behavioral test of impulsivity in the involving choice between an immediate, smaller reward and a delayed, larger one (Bizot et al. 1999). Beginning 1 week after dorsal raphe nucleus lesioning with 5,7-DHT, lesioned rats were more likely to choose the smaller, immediate reward when the larger reward was delayed by 15 seconds. This difference was only detected 7 to 8 days after lesioning and not subsequently. 24 days after lesioning, tryptophan hydroxylase activity was found to be 36% of controls in the cerebral cortex and hippocampus, confirming that extensive neurotoxicity had occurred. In another study, dorsal raphe nucleus lesioning with 5,6-DHT did not influence passive avoidance behavior in rats at 10 days post-lesioning, despite a reduction of dorsal hippocampal SERT activity to 20% of controls (Kovacs et al. 1979). However, lesioned rats were insensitive to microinjection of arginine-vasopressin into the dorsal raphe nucleus, a treatment that normally improves consolidation of learning. In summary, lesioning of the dorsal raphe serotonergic system is associated with transient behavioral changes that could be interpreted as increased interest in novel environments and more impulsive behavior.

Because lesioning of the dorsal raphe serotonergic system with established neurotoxins is associated with subtle and often transient effects, it does not appear plausible to draw strong conclusions from the difficulty researchers have had associating MDMA neurotoxicity with behavioral alterations. Similarly, strong conclusions cannot be drawn from studies directly comparing the behavioral effects of MDMA and DHT neurotoxicity if DHT lesions were not limited to the dorsal raphe nucleus. Although three studies (Lorens et al. 1989; Ricaurte et al. 1993; Seiden et al. 1993) detected behavioral effects of 5,7-DHT but not MDMA, 5,7-DHT was given intracerebroventicularly in all cases and likely produced a different pattern of serotonergic damage than MDMA.

How Can Neurotoxic Damage be Without Detectable Behavioral Consequences?

Two concepts help to explain why neurodegeneration or neurotoxic damage is not always associated with predictable behavioral effects. First, there is a threshold of damage that must be exceeded in some brain systems before symptoms develop. This has been primarily investigated with dopaminergic cell loss and Parkinson's disease (Brownell et al. 1999; Calne et al. 1985; Di Monte et al. 2000). There are less data on the serotonergic system. A rat study using the serotonergic neurotoxin, 5,7-DHT (Kirby et al. 1995) found that basal extracellular 5HT levels in the ventral striatum were not altered in rats with striatal 5HT levels reduced to 3.1% of controls. In contrast, Hall et al. (1999) concluded that a loss of greater than 60% of serotonergic neurons was necessary to decrease extracellular 5HT levels in the striatum. Alterations in behavior were seen with slightly smaller depletions (51% or more), possibly due to regional variations in neurotoxicity. Hall et al. suggest that the previous study failed to find decreases in extracellular 5HT levels because the recently implanted microcanulae may have damaged cells, increasing extracellular 5HT. One might speculate that even smaller depletions may not affect many serotonergic-related behaviors, although the maximal serotonergic

response to drugs or other stimuli is likely to be reduced (reduced electrical-stimulated 5HT release in MDMA-exposed rats was documented by (Gartside et al. 1996). Another concept - called cognitive reserve - has been developed to explain why greater education, intelligence, or brain size is associated with less severe impairment in conditions such as Alzheimer's disease, AIDS, and normal aging (Alexander et al. 1997; Coffey et al. 1999; Graves et al. 1996; Stern et al. 1996). This cognitive reserve may be seen as a surplus of processing capacity that protects the individual against loss of functioning when processing capacity is decreased.