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LONG-TERM EFFECTS OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE
(MDMA) ON SEROTONERGIC AND DOPAMINERGIC FUNCTIONING

A Thesis
Presented to the
Faculty of
California State University,
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts in
Psychology:
General-Experimental

by
Jodi Lynn Kohutek
September 2003
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ABSTRACT

3,4-Methylenedioxymethamphetamine (MDMA) is a popular recreational drug that has been shown to increase serotonin and dopamine levels, yet little is known about its long-term effects in brain. Recently, however, studies examining the effects of prenatal MDMA exposure on brain functioning have indicated that MDMA exposure may be responsible for long-term cognitive deficits. Therefore, in the present study we administered MDMA (20 mg/kg twice per day) to both male and female preweanling rats on postnatal days (PD) 11-20, to assess whether MDMA caused permanent deficits in serotonergic and dopaminergic functioning. We measured serotonin, 5-hydroxyindoleacetic acid, dopamine, dihydroxyphenyleacetic acid levels, and PKA activity in the striatum, prefrontal cortex, and hippocampus on PD 90. MDMA pre-exposure caused significant reductions in serotonin concentrations in both the prefrontal cortex and hippocampus. In addition, the striatum and prefrontal cortex showed significant reductions in dopamine, however only in the prefrontal cortex were significant declines in dihydroxyphenyleacetic acid levels observed. MDMA pre-exposed rats showed a significant decline in protein kinase A activity in both prefrontal cortex and hippocampus. When considered
together, this study provides the first evidence that exposure to MDMA during the preweanling period has long-term neurotoxic effects on both serotonergic and dopaminergic systems.
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CHAPTER ONE
INTRODUCTION

3,4-Methylenedioxymethamphetamine (MDMA) Overview

3,4-Methylenedioxymethamphetamine (MDMA), popularly known as "Ecstasy", continues to gain popularity as a recreational drug (Cregg & Tracey, 1993; Cuomo, Dyment, & Gammino, 1994; Ricaurte, Finnegan, Irwin, & Langston, 1990), despite compelling evidence that MDMA is a potent toxic compound affecting both brain serotonergic (Green, Cross, & Goodwin, 1995; Ricaurte, Byran, Stauss, Seiden, & Schuster, 1985) and dopaminergic functioning (Hotchkiss & Gibb, 1980; Ricaurte, Schuster, & Seiden, 1980; Seiden, Fischman, & Schuster, 1975). Illicit use of MDMA did not become popular until the late 1980's among college campuses and underground "rave" parties. The incidence of MDMA usage has escalated rapidly, largely due to its euphoria-inducing and mild stimulant properties. Although first synthesized as a pharmacotherapeutic agent in the early 1900's (Shulgin, 1986), it was quickly abandoned until the 1970's, when some mental health professionals began using it in an attempt to facilitate psychotherapy (Greer, 1985; Greer & Tolbert, 1986; Shulgin & Nichols, 1978). Even though both the therapeutic communities and
recreational drug users displayed intense interest in
MDMA, the Drug Enforcement Administration (DEA)
categorized MDMA as a Schedule I synthetic substance
(Lawn, 1986). Schedule I drugs have a high potential for
abuse and are not accepted for medical use in the United
States.

Due to the drastic increase in the abuse of MDMA,
researchers have begun to intensively investigate the
neurobiological effects of MDMA on various neuronal
parameters. Researchers using animals have shown that MDMA
affects motor, autonomic, and general central nervous
system (CNS) functioning (Shulgin, 1986). These effects
are similar to those observed in humans and include
hyperactivity, excitability, aggressive behavior,
convulsions, muscular rigidity, mydriasis, and salivation
(Hardman, Haavik, & Seevers, 1973).

Evidence that MDMA causes brain damage is based on
experiments involving non-primates and primates. For
example, both acute and chronic treatment with MDMA
produces significant damage to serotonin (5-HT) neurons in
rat brain (Battaglia, Yeh, & De Souza, 1988; Ricaurte,
DeLanney, Irwin, & Langston, 1988). MDMA also alters
serotonergic functioning in primate brain, as this drug
causes regional decreases in 5-HT metabolites (e.g.,
5-hydroxyindoleacetic acid; 5-HIAA) and a reduction in 5-HT uptake sites (Insel, Battaglia, Johannessen, Marra, & De Souza, 1989; Ricaurte et al., 1988). MDMA also disrupts dopaminergic functioning by altering the activity of dopamine (DA) transporters, and by reducing both tyrosine hydroxylase (TH) activity and DA levels (Hotchkiss & Gibb, 1980; Metzer, Hanson, Gibb, & Fleckenstein, 1998; Nash & Nichols, 1991; Wagner, Ricaurte, Seiden, Schuster, Miller, & Westley, 1980). Decreased dopaminergic and serotonergic neurotransmission has been implicated in cognitive impairment and numerous psychiatric disorders (Kahn & Davis, 1994; Maes & Meltzer, 1994); thus, it is important to determine the neural mechanisms affected by MDMA exposure.

Summary and Proposal

It appears that MDMA induces neurotoxicity in serotonergic and dopaminergic neurons. The purpose of this project is to assess the long-term effects of repeated administration of MDMA on both serotonergic and dopaminergic neuronal functioning. Specifically, male and female rats will be given daily administrations of MDMA (50 mg/kg twice per day) on postnatal days (PD) 11-20. Rats will be killed at PD 90 and samples from the dorsal striatum, prefrontal cortex, and hippocampus will be
assayed for 5-HT, 5-HIAA, DA, dihydroxyphenyleacetic acid (DOPAC) levels, and protein kinase A (PKA) activity. Measuring these neurotransmitters (i.e., 5-HT and DA) is a common method for assessing neurotoxicity. In addition, PKA will be measured because it is an important component of second messenger systems associated with both 5-HT and DA, and PKA activity is reduced after repeated treatments with amphetamine and methamphetamine (Crawford, Zavala, Karper, Collins, Loring-Meier, Watson, & McDougall, 2000a; Terwilliger, Beitner-Johnson, Sevarino, Crain, & Nestler, 1991). Thus, this study will attempt to determine whether early exposure to MDMA causes neurotoxicity in 5-HT and DA neurons or a decline in PKA activity.

Serotonin Systems

Neuropharmacology

5-HT is a monoamine neurotransmitter found in both the CNS and peripheral nervous system (PNS). 5-HT plays a variety of roles in neurological functioning. More specifically, 5-HT has been implicated in the etiology or treatment of various disorders, such as anxiety, depression, schizophrenia, and obesity (Naughton, Mulrooney, & Leonard, 2000). Serotonergic neurons are principally located in several clusters along the midline
or the raphe of the brainstem. The dorsal raphe is situated in the midbrain central gray matter on the midline just below the sylvian aqueduct and the median raphe is located ventrally on the midline (Feldman, Meyer, & Quenzer, 1997).

5-HT is synthesized in a two-step process from the essential amino acid tryptophan. Tryptophan is the rate-limiting step in the biosynthesis of 5-HT; therefore, the amount of tryptophan in the brain determines the amount of 5-HT produced. After tryptophan enters the presynaptic terminal the enzyme tryptophan hydroxylase converts tryptophan to 5-hydroxytryptophan (5-HTP). Amino acid decarboxylase removes a carboxyl group from 5-HTP to yield 5-HT (Cox & Nelson, 2000). Free-floating 5-HT is rapidly metabolized by MAO and the resulting substance is readily converted by aldehyde dehydrogenase to 5-HIAA. Thus, 5-HIAA is the major metabolite of 5-HT. 5-HT is removed from the synaptic cleft by either an active reuptake process or by MAO. Like the dopaminergic reuptake system, Na+/Cl⁻ is necessary for the 5-HT transporter to function (Graham, Esnaud, Habert, & Langer, 1989; Marcusson & Ross, 1990).

Molecular biology techniques have paved the way for tremendous progress in 5-HT receptor identification. 5-HT
can act presynaptically or postsynaptically, and can be either excitatory or inhibitory. Thus, it is no surprise that more than 16 different 5-HT receptors have been identified (Cryan & Leonard, 2000; Dubovsky & Thomas, 1995; Teitler & Herrick-Davis, 1994). Currently, 5-HT receptors are classified according to their structure into three major families of receptors: 5-HT₁, 5-HT₂, and 5-HT₃. These families of 5-HT receptors can be further subdivided into a variety of receptor subtypes [e.g., 5-HT₁A, 5-HT₁B, etc.] (Bradley, Engel, Feniuk, Fozard, Humphrey, Middlemiss, Mylechrane, Richardson, & Saxena, 1986; Zifa & Fillion, 1992). With the exception of the 5-HT₃ receptor, which is a ligand-gated ion channel, all other 5-HT receptor subtypes are G-protein coupled receptors (Teitler & Herrick-Davis, 1994). 5-HT₁ receptors have a high affinity for 5-HT and stimulation of these receptors inhibits adenylase cyclase activity and opens a K⁺ channel producing hyperpolarizations (Chen & Penington, 1996, 1997; Penington & Fox, 1995; Penington, Kelly, & Fox, 1993; Premkumar & Ahern, 1995). 5-HT₂ receptors are low-affinity receptors and activation of these receptors can either increase Ca²⁺ levels by stimulating phosphoinositol hydrolysis or depolarize neurons by closing K⁺ channels (Chen & Penington, 1997, 1996;
Penington & Fox, 1995; Penington et al., 1993; Premkumar & Ahern, 1995).

Neuroanatomy and Behavior

Sero tonergic neurons originate in the brain stem and project throughout the brain. More specifically, 5-HT neurons can be divided into two distinct systems: the caudal system and the rostral system (Sanders-Bush & Canton, 1995; Tork, 1990). The caudal system, which is consistent with B1-B4 of Dahlstrom and Fuxe (1964), originates in the median and paramedian medulla and caudal pons, with axons terminating in the spinal cord (Tork, 1990). These descending pathways are involved in sensory, motor, and autonomic functioning (Whitaker-Azmitia, 1991). The rostral system, which is consistent with the B5-B9 cell groups (Dahlstrom & Fuxe, 1964), originates in raphe nuclei of the rostral pons, mesencephalon, caudal linear nucleus, nucleus pontis oralis, and the supramammical region (Nieuwenhuys, 1985; Tork, 1990). Fibers projecting from these neurons ascend to the diencephalon, basal ganglia, limbic system, and cortex via two distinct fiber tracts: the ventral and dorsal ascending pathways (Nieuwenhuys, 1985).
Dopamine Systems

Neuropharmacology

DA was initially considered to be merely an intermediate step in the synthesis of norepinephrine and epinephrine. In the late 1950's, however, DA was discovered to be a neurotransmitter involved in the control of motor movement and emotional behaviors (Seeman, 1995). DA is a catecholamine neurotransmitter synthesized in nerve terminals in the hypothalamus, arcuate nucleus, caudate, nucleus accumbens, prefrontal cortex, hippocampus, and various other areas of the CNS (Feldman et al., 1997; Wolf & Roth, 1987). DA is impermeable to the blood brain barrier, thus it must be synthesized in the CNS (Cooper, Bloom, & Roth, 1996). Specifically, tyrosine readily passes the blood-brain barrier where it is actively absorbed into the brain. Upon entry into DA terminals, tyrosine is converted to L-dihydroxyphenylalanine (L-DOPA) by the enzyme TH. The rate at which this conversion takes place is limited by the amount of TH present, thus TH is the rate-limiting step in DA synthesis. L-DOPA is then quickly converted to DA by the enzyme DOPA decarboxylase (Roth & Elsworth, 1995).
After being released into the synaptic cleft, the DA transporter, a Na+/Cl- dependent mechanism, transports DA in either direction depending on the concentration gradient (Krueger, 1990). Thus, in the normal case DA is transported from the synaptic cleft to the presynaptic terminals where it is either repackaged into vesicles or metabolized into DOPAC by the enzyme monoamine oxidase (MAO). Extracellular DA can be metabolized by the enzyme catechol-0-methyltransferase (COMT) and converted into homovanillic acid (HVC), one of the primary metabolites of DA (Kopin, 1985).

DA can inhibit or excite other neurons, depending on the properties of the receptor stimulated. There are two families of DA receptors differentiated on the basis of their pharmacology: D_{1}-like and D_{2}-like receptors (Civelli, 1995; Kebabian & Calne, 1979). These two families of receptors include five structurally distinct subtypes: D_{1}, D_{2}, D_{3}, D_{4}, and D_{5} receptors (Cooper et al., 1996). The D_{1} and D_{5} receptors (i.e., D_{1}-like receptors) are closely related, as they stimulate adenylyl cyclase activity, increase cyclic AMP (cAMP), and are excitatory. In contrast, D_{2}, D_{3}, and D_{4} receptors (i.e., D_{2}-like receptors) inhibit adenylyl cyclase activity, decrease
cAMP activity, and are inhibitory (Andersen, Gingrich, Bates, Deary, Falardeau, & Senogles, 1990).

**Neuroanatomy and Behavior**

Dopaminergic neurons are found in small and large circuit pathways in the CNS. The nigrostriatal pathway, one of the larger pathways, is the primary DA pathway projecting from the substantia nigra and terminating in the caudate and putamen (Bannon & Roth, 1983). Degeneration of dopaminergic neurons in the nigrostriatal pathway is responsible for motor deficits associated with Parkinson’s disease (Bannon & Roth, 1983; Cooper et al., 1991). DA neurons also compose the mesolimbic and mesocortical pathways, which originate in the ventral tegmental area (VTA) and project to parts of the limbic system and cortex, respectively. DA neurons in the limbic system modulate both locomotor activity and motivation (LeMoal & Simon, 1991); whereas, DA neurons projecting to the cortex appear to be involved in cognition and emotional behavior (Wolf, Deutch, & Roth, 1987). Excess dopaminergic transmission within the mesolimbic and mesocortical systems has been linked to schizophrenia (Kahn & Davis, 1994), as well as some of the motor and attention problems associated with Tourette’s disorder and attention deficit hyperactivity disorder [ADHD] (Comings,
Behavioral Impact of MDMA Exposure

A substantial number of studies using various species have examined the behavioral effects induced by MDMA. While many studies have assessed behavioral changes induced by acute MDMA treatment other studies have examined the cognitive impact of repeated MDMA exposure.

Acute Behavioral Effects of MDMA

The acute behavioral effects characteristic of MDMA treatment are caused by a rapid decline in TPH and reduced 5-HT activity, coupled with increased DA release. Behavioral effects of acute MDMA treatment have been assessed through a variety of paradigms, including the elevated plus-maze, open field, skilled paw-reach tasks, and threat and attack paradigms. Paradigms designed to assess anxiety suggest that MDMA's effects are complex. For example, on the elevated plus-maze, lower doses of MDMA (4 mg/kg) produce an anxiogenic effect in mice, whereas at higher doses (20 mg/kg) anxiolytic effects are
apparent (Lin, Burden, Christie, & Johnson, 1999). In contrast, rats given medium to high doses of MDMA display an anxiogenic response when assessed on an elevated plus-maze (Bhattacharyya, Bhattacharyya, & Ghosal, 1998). When assessed in the open field, rodents treated with MDMA exhibit a decrease in anxiety-related behaviors and an increase in locomotor activity (Mechan, Moran, & Elliot, 2002). Significant impairments in spontaneous locomotor activity and skilled motor function are also observed after MDMA exposure (Marston, Reid, Lawrence, Oliverman, & Butcher, 1999). Lastly, MDMA reduces aggressive behaviors in rodents when assessed on threat and attack paradigms (Miczek & Haney, 1994). The latter findings are interesting, because reductions in 5-HT activity typically increase aggressive behaviors in rodents.

In addition to examining the unlearned effects of MDMA exposure, a number of laboratories have examined whether MDMA causes cognitive impairments (Bolla, McCann, & Ricaurte, 1998; Curran & Travill, 1997; Morgan, 1999). Various tests of memory function (e.g., delayed non-match to place and self-ordered spatial search tasks) have demonstrated that acute MDMA treatment induces cognitive deficits (Marston et al., 1999; Taffe, Weed, Davis, Huitron-Resendiz, Schroeder, Parsons, Henriksen, & Gold,
More specifically, on the delayed non-match to place task, rats treated with MDMA display severe memory impairments (Marston et al., 1999). In addition, acute MDMA treatment causes a dose-dependent decrease in both accuracy and response rates on a delayed non-match to sample task in pigeons (LeSage, Clark, & Poling, 1993). Rhesus monkeys perform poorly on cognitive battery measures after acute treatment with MDMA (Taffe et al., 2001). These alterations in both unlearned behaviors and cognitive functioning are credited to neurochemical changes caused by acute MDMA treatment.

**Long-Term Behavioral Effects of MDMA**

It has been well documented that administration of MDMA, either in a single high dose or multiple doses, produces long-term damage to 5-HT nerve terminals, but the long-term behavioral impact of MDMA neurotoxicity remains unclear (Molliver, Berger, Mamounas, Molliver, O’Hearn, & Wilson, 1990; O’Hearn, Battaglia, De Souza, Kuhar, & Molliver, 1988; O’Shea, Granados, Esteban, Colado, & Green, 1998). The neurotoxic effects of MDMA exposure first appear between 1 and 7 days after drug administration and are characterized by a decrease in 5-HT concentrations, loss in functional 5-HT uptake sites, and degeneration of serotonergic terminals (McKenna &
Brain levels of DA and its metabolites are not affected by low doses of MDMA, but are depleted after higher doses. This pattern of results suggests that MDMA is capable of inducing DA neurotoxicity, and that MDMA is more toxic to 5-HT than DA neurons (Commins, Vosmer, Virus, Woolverton, Schuster, & Seiden, 1987; Laverty & Logan, 1990; Miller & O’Callaghan, 1995).

There is some evidence, although it is not conclusive, that MDMA exposure has long-term impact on behavioral and cognitive performance. On the elevated plus-maze and open field tasks, MDMA-treated rodents exhibit reduced anxiety and increased risk-taking behavior (Mechan et al., 2002). However, when behavior is assessed using a social interaction paradigm, rats given MDMA have fewer social interactions than their controls (Fone, Beckett, Topham, Swettenham, Ball, & Maddocks, 2002), suggesting that MDMA causes increased social anxiety (File, 1980). Studies using rodents indicate that MDMA exposure causes long-term cognitive deficits. For example, rats treated with MDMA display deficits on a delayed non-matching to position working memory task for up to 19 days after drug treatment (Marston et al., 1999). This cognitive deficit, which suggests that MDMA impairs
short-term memory, is consistent with the finding that MDMA causes degeneration of serotonergic neurons in the cortex and hippocampus (Marston et al., 1999). In contrast, the performance of rats on a delayed alternation task was generally unaffected by neurotoxic doses of MDMA (Ricaurte, Markowska, Wenk, Hatzidimitriou, Wlos, & Olton, 1993). Consistent with the latter study, primates display an absence of learning impairments after exposure to MDMA (Frederick, Ali, Slikker, Gilliam, Allen, & Paule, 1995). Therefore, while it has been well documented that MDMA-induces neurotoxicity, much remains to be discovered about the behavioral consequences that accompany long-term depletion of 5-HT and DA.

It is uncertain whether MDMA exposure causes neurotoxicity in humans, but emerging evidence indicates that repeated MDMA exposure results in performance decrements in neurocognitive functioning (Morgan, 1999; Rodgers, 2000). The manifestations of these deficits, in terms of altered cerebral functioning and behavioral changes, range from neuroendocrine impairments to deficits in verbal memory and reasoning, short-term memory, semantic recognition, and visual memory (Broening, Morford, Imman-Wood, Fukumura, & Vorhees, 2001; Marston et al., 1999; Morgan, 1999; Rodgers, 2000). Therefore,
despite some conflicting reports, there is ample evidence that MDMA exposure has long-term behavioral impact on both animals and humans. Understanding the nature of this MDMA-induced neurotoxicity may be important for the treatment of personality disorders, impulsive/aggressive behavior, and learning impairments.

**MDMA-Induced Neurotoxicity: Serotonin Systems**

MDMA exposure causes characteristic patterns of neurotoxicity, as MDMA exerts marked effects on serotonergic systems in brain (Battaglia, Yeh, O’Hearn, Molliver, Kuhar, De Souza, 1987; Schmidt, 1987; Sprague, Everman, & Nichols, 1998; White, Obradovic, Imel, & Wheaton, 1996). For example, neurochemical studies in rodents and primates have demonstrated that MDMA exposure results in a profound decrease in 5-HT concentrations (Battaglia et al., 1987; Commins et al., 1987; Stone, Stahl, Hanson, & Gibb, 1986). In addition, MDMA exposure reduces 5-HT transporters and synaptosomal uptake activity (Stone et al., 1986), decreases the density of 5-HT axon terminals (Battaglia et al., 1987; Huether, Zhou, & Ruther, 1997; O’Hearn et al., 1988), and causes declines in 5-HIAA and TPH levels (Battaglia et al., 1987; Schmidt, 1987; Stone et al., 1986). As mentioned previously, TPH is
the rate-limiting step in 5-HT synthesis, and reductions in brain TPH activity may be one of the initial neurotoxic effects produced by MDMA (Schmidt & Kehne, 1990).

The neurotoxic effects of MDMA exposure are often quantified by assessing reductions in transporter site density using $[^3H]$-paroxetine (Battaglia et al., 1987). In addition, neurotoxic damage to 5-HT nerve terminals can be determined by measuring decreases in 5-HT and 5-HIAA levels (Battaglia et al., 1987; Hewitt & Green, 1994; Stone et al., 1986). Advanced brain imaging techniques, such as positron emission tomography (PET), can also be used to assess neuronal damage. For example, PET imaging indicates that MDMA exposure causes significant reductions in the number of 5-HT transporters in various brain regions (Commins et al., 1987; McCann, Szabo, Scheffel, Dannals, & Ricaurte, 1998). Most typically, damage to 5-HT neurons has been demonstrated in the hippocampus, neocortex, striatum, thalamus, and amygdala (Molliver et al., 1990; O'Hearn et al., 1988).

**Acute MDMA Exposure**

Acute treatment with MDMA can induce neurotoxicity, but the extent of the damage varies according to dose. Evidence of neuronal damage has been revealed by the application of silver staining techniques, which have
shown that a single exposure to MDMA (40 mg/kg) produces a substantial increase (90%) in the number of degenerating axons and nerve terminals (Commins et al., 1987; Slikker et al., 1988). Similarly, a single injection of 20 or 40 mg/kg MDMA is capable of inducing 5-HT toxicity in the striatum, hippocampus, and cortex; however, neurotoxicity in the hypothalamus was only induced by an injection of 40 mg/kg MDMA (Commins et al., 1986). Acute treatment with a lower dose (10 mg/kg) of MDMA caused a significant decline in the density of 5-HT uptake sites in prefrontal cortex of rat brain (Battaglia et al., 1988). Likewise, a single injection of 10, 15, or 20 mg/kg MDMA caused a significant decline in TPH activity and an increase in transporter-mediated 5-HT in the neostriatum, hippocampus, and hypothalamus of adult rats (Battaglia et al., 1988; Ricaurte et al., 1988; Schmidt & Kehne, 1990; Stone, Merchant, Hanson, & Gibb, 1987), while an acute injection of 15 mg/kg MDMA reduced 5-HT and 5-HIAA levels by more than 50% in the cortex, hippocampus, nucleus accumbens and striatum (Mayerhofer, Kovar, & Schmidt, 2001; O’Shea et al., 1998). 5-HT neurons in the cerebral cortex of monkey brain also show structural changes and axonal damage after exposure to 5 mg/kg MDMA (Ricaurte et al., 1988). Therefore, it is clear that an acute injection of a
moderate to high dose (i.e., 10-40 mg/kg) of MDMA causes substantial 5-HT neurotoxicity; whether lower doses of MDMA induce neurotoxicity is more uncertain.

Chronic MDMA Exposure

Repeated administration of MDMA causes 5-HT neurotoxicity in animals. MDMA-induced neurodegeneration appears to be dosage- and frequency-dependent. Rats treated with multiple low doses of MDMA (4 mg/kg), either once daily over a four day period or twice weekly over an eight week period, show no evidence of 5-HT neurotoxicity (O’Shea et al., 1998). When the same study was replicated with slight alterations in the frequency of MDMA exposure (4 mg/kg twice daily for a four day period) significant reductions in 5-HT uptake sites were reported (O’Shea et al., 1998). In rhesus monkeys, multiple injections with a low dose of MDMA (2.5 mg/kg twice daily for four days) reduced 5-HT levels in the hippocampus (Ali, Newport, Scallet, Binienba, Ferguson, Bailey, Paule, & Slikker, 1993). This is of particular interest because squirrel monkeys exposed to 2.5 mg/kg MDMA twice a month for four months showed no long-term changes in 5-HT or 5-HIAA levels or 5-HT transporter sites (Ricaurte et al., 1988). More frequent treatment with MDMA (5 mg/kg twice daily for four days) produces substantially more neurotoxicity in
monkey brain, as immunostaining indicated that serotonergic axons were swollen and fragmented (Ricaurte et al., 1988). Based on these studies, it appears that the effects of MDMA are both dosage and frequency dependent, with larger doses and more frequent drug administrations producing greater neurotoxicity (Ali et al., 1993; Battaglia et al., 1988; O'Shea et al., 1998; Ricaurte et al., 1988).

Similarly, studies have reported a decrease in serotonergic functioning with multiple injections of higher doses of MDMA. Repeated treatment with 10-40 mg/kg MDMA reduces THP activity, 5-HT levels, and 5-HIAA levels by at least 50-60% in the neostriatum, hippocampus, and cortex of rat brain (Battaglia et al., 1987; Commins, 1986; Gibb et al., 1990). Likewise, rats given eight consecutive injections of 20 mg/kg MDMA over a four day period demonstrated up to a 76% depletion of 5-HT transporters in the prefrontal cortex, hippocampus, striatum, hypothalamus, and midbrain (Battaglia et al., 1987; Obradovic, Imel, & White, 1996; Schmidt, 1994). Thus, there is an abundance of data showing that repeated treatment with moderate to high doses of MDMA causes marked 5-HT neurotoxicity and depressed 5-HT functioning (Gibb et al., 1990).
MDMA-Induced Neurotoxicity: Dopamine Systems

Several in vivo and in vitro studies have shown that MDMA robustly evokes the release of DA and induces DA neurotoxicity (Nash & Nichols, 1991; Nash & Yamamoto, 1992; Shankaran & Gudelsky, 1998; Yamamoto & Spanos, 1988). Furthermore, it has been suggested that there is an intimate relationship between DA release and the long-term depletion of 5-HT (Nash & Nichols, 1991). That is, MDMA releases DA through two separate mechanisms: first, by directly releasing DA via the transporter (Johnson, Hoffman, Nichols, 1986; Schmidt, Levin, & Lovenburg, 1987) and, second, by indirectly releasing DA through actions on 5-HT systems [the release of 5-HT activates 5-HT receptors which stimulates DA synthesis and release] (McCann & Ricaurte, 1993; Nash, 1990). Evidence for the first mechanism of action is provided by studies showing that selective DA inhibitors (i.e., GBR 12909 and \(\alpha\)-methyl-\(\beta\)-tyrosine) suppress MDMA-induced DA and 5-HT release (Brodkin, Malyala, & Nash, 1993; Stone, Johnson, Hanson, & Gibb, 1988). Although MDMA causes only modest depletions of DA, relative to 5-HT, MDMA does induce significant DA neurotoxicity in the hippocampus, nucleus accumbens, striatum, and other regions of the brain.
Acute MDMA Exposure

Researchers have found that MDMA-induced neurotoxicity not only requires 5-HT release, but also DA efflux from cells (Rudnick & Wall, 1993). Acute treatment with low to moderate doses of MDMA (1-20 mg/kg) causes a dose-dependent elevation of extracellular DA levels in the nucleus accumbens and hippocampus (Kankaanpaa, Meririnne, Lillsunde, & Seppala, 1998; Shankaran & Gudelsky, 1998). This is of particular interest, because extracellular DA promotes free radical formation and inhibits glutamate-evoked firing: two mechanisms responsible for neurotoxicity (Cadet, Ladenheim, Hirata, Rothman, Ali, Carson, Epstein, & Morgan; 1995; Colado, O'Shea, Grandos, Misra, Murray, & Green, 1997; Obradovic et al., 1996; Shankaran, Yamamoto, & Gudelsky, 1999; White, Duffy, & Kalivas, 1994; Yamamoto & Spanos, 1988). In addition, rats exposed to MDMA (1.0, 3.0, or 9.0 mg/kg) show significantly depressed DOPAC concentrations, however HVA concentrations were only reduced after an acute injection of 9.0 mg/kg MDMA (Kankaanpaa et al., 1998). Curiously, an earlier study reported that moderate to high doses (10, 20, or 40 mg/kg) of MDMA does not cause DA neurotoxicity
in any of the brain regions examined (Commins et al., 1986). These results are consistent with other reports indicating that MDMA, given at doses which induce 5-HT neurotoxicity, leaves the DA system virtually unaltered (Battaglia et al., 1987; Colado, O'Shea, Granados, Esteban, Martin, & Green, 1999; Schmidt & Kehne, 1990). Due to these conflicting results, it remains uncertain whether acute MDMA exposure produces measurable DA neurotoxicity.

**Chronic MDMA Exposure**

In contrast to the acute administration literature, there is more consistent evidence that repeated treatment with high doses of MDMA causes DA neurotoxicity (Green et al., 1995; Logan, Laverty, Sanderson, & Yee, 1988). Although dopaminergic toxicity has been evidenced in both primates and non-primates, many of the DA toxicity studies use a mouse model (Johnson, O’Callaghan, & Miller, 2002; Johnson, Shvedova, Kisin, O’Callaghan, Kommineni, & Miller, 2002; O’Shea, Esteban, Camarero, Green, & Colado, 2001). More specifically, mice treated with MDMA show long lasting depletions of striatal DA, HVA, DOPAC, and TH (O’Callaghan & Miller, 1994).
Factors Affecting Neurotoxicity

Protection from Serotonergic and Dopaminergic Neurotoxicity

In vivo and in vitro studies have utilized selective inhibitors of monoamine transporters to analyze the effects of MDMA on serotonergic and dopaminergic neuronal systems. Brain dialysis studies have shown that pretreating rats with dopaminergic receptor antagonists (e.g., haloperidol), DA depletive agents (α-methyl-ρ-tyrosine), selective DA transport inhibitors (e.g., GBR 12909), or selective 5-HT transport inhibitors (e.g., fluoxetine) will prevent MDMA-induced neurotoxicity to DA and 5-HT neurons (Bradberry, 1994; Brodkin et al., 1993; Colado et al., 1999; Green et al., 1995; Schmidt et al., 1991; Stone et al., 1988). For example, rats pretreated with haloperidol show an attenuated loss of 5-HT transporter sites, suggesting that DA release contributes to 5-HT transporter destruction (Hewitt & Green, 1994). Furthermore, GBR-12909 not only inhibits MDMA-induced DA release but attenuates 5-HT neurotoxicity (Koch & Galloway, 1997). MDMA-induced 5-HT neurotoxicity can also be reduced by serotonergic antagonists [fluoxetine, WAY 100135, and methiothepin] (Obradovic et al., 1996). For example, pretreatment with selective 5-HT
reuptake inhibitors [SSRI's] (e.g., fluoxetine or
citalopram) block both MDMA-induced 5-HT release and
subsequent toxicity (Bradberry, 1994; Gudelsky & Nash,
1996; Schmidt, 1987). Thus, when the release of DA and
5-HT are blocked by antagonist drugs, the ability of MDMA
to induce neurotoxicity is significantly attenuated.

Effects of Ambient Temperature

Researchers have also investigated the influence of
ambient temperature on MDMA-induced neurotoxicity, as it
has been repeatedly shown that ambient temperature is a
critical factor in determining the extent of MDMA-induced
neurotoxicity (Broening, Bowyer, & Slikker, 1995; Malberg
& Seiden, 1998; Russel & Laverty, 2001). For example,
MDMA-treated rats maintained at a lower ambient
temperature (20° or 22° C) display no 5-HT neurotoxicity,
whereas MDMA-treated rats maintained at a higher ambient
temperature (26°, 28°, or 30° C) show significant
decreases in 5-HT and 5-HIAA levels (Malberg & Seiden,
1998). Ambient temperature probably plays a vital role in
determining the extent of toxicity because hyperthermia is
a mechanism responsible for neuronal death (Bowyer, Gough,
Slikker, Lipe, Newport, & Holson, 1993; Bowyer, Tank,
Newport, Slikker, Ali, & Holson, 1992). Thus, hyperthermia
is critical for MDMA-induced neurotoxicity, and this
neurotoxicity can be attenuated by either lowering core ambient temperature or by coadministering drugs that produce a hypothermic response (Hewitt & Green, 1994; Malberg et al., 1996).

MDMA-Induced Effects on Behavior and Neural Development

Prenatal Drug Exposure

Several teratological studies have attempted to identify possible adverse effects caused by prenatal drug exposure. This is of importance because clinical studies have reported that human infants exposed prenatally to psychostimulant drugs exhibit many neurobehavioral problems, such as increased irritability, impairments in orientation and motor ability reflexitivity, and depression of interactive behavior (Chasnoff, Burnes, Schnoll, & Burnes, 1985; Chasnoff, Griffith, MacGregor, Derkes, & Burnes, 1989; Chasnoff & Lewis, 1987). Animal models have shown long-term alterations in behavior after in utero drug exposure. These alterations include modifications in locomotor activity and deficits in information acquisition (Heyser, Chen, Miller, Spear, & Spear, 1990). For instance, one study reported that prenatal cocaine exposure results in long-lasting alterations in brain metabolism, including decreased
glucose utilization along with increased D₁ receptor concentrations in the substantia nigra of adult male offspring (Dow-Edwards, Freed, & Fico, 1990).

Surprisingly, relatively few studies have examined the effects of prenatal MDMA exposure on brain functioning (Aguirre, Barrionuevo, Lasheras, & Del Rio, 1998; Colado et al., 1997; St. Omer, Ali, Holson, Duhart, Scalzo, & Slikker, 1991). Although MDMA induces neurotoxicity in adult rats, results indicate that administering MDMA during gestation has no effect on postnatal neurochemical development of the 5-HT system (St. Omer et al., 1991). More specifically, pregnant rats administered varying doses of MDMA (2.5 and 10.0 mg/kg on alternate gestational days 6-18) had offspring with normal numbers of 5-HT transporters, and 5-HT and 5-HIAA levels remained unaffected (St. Omer et al., 1991). This is of interest because MDMA caused significant reductions in the 5-HT and 5-HIAA levels of the dams (St. Omer et al., 1991).

When all of these results are considered together, it is surprising that MDMA exposure does not affect neonates. One possibility is that MDMA lacks impact because neonates have underdeveloped transporter mechanisms (St. Omer et al., 1991). Alternatively, extracellular DA levels may not be great enough to produce oxidative stress in 5-HT
terminals (Aguirre et al., 1998) or neonates may have a higher capacity to scavenge free radicals (Colado et al., 1997; Sprague et al., 1998). In any event, it appears that in utero MDMA exposure does not induce measurable neurotoxicity in rats.

Postnatal Drug Exposure

Studies examining the neurochemical consequences of MDMA exposure during the early postnatal period are sparse. However, most studies suggest that preweanling rats, like neonates, are resistant to MDMA-induced neurodegeneration (Aguirre et al., 1998; Broening et al., 1994). In contrast, a recent report indicates that early postnatal MDMA exposure does indeed have long-term cognitive effects (Broening et al., 2001). The study focused on two dosing periods (postnatal day [PD] 1-10 and PD 11-20) and four doses of MDMA (0, 5.0, 10.0, and 20.0 mg/kg, given twice daily). Results indicated that MDMA exposure on PD 11-20 significantly disrupted both sequential and spatial learning of rats tested on a Multiple-T water maze (Broening et al., 2001). More specifically, PD 11-20 rats exhibited consistent deficits in sequential learning, measured in terms of errors and escape latencies on the Multiple-T water maze, whereas rats given MDMA on PD 1-10 did not show any behavioral
deficits (Broening et al., 2001; Vorhees, Ahrens, Acuff-Smith, Schilling, & Fisher, 1994). Therefore, it is apparent that repeated treatment with MDMA during the preweanling period has long-term behavioral impact. The neural mechanisms responsible for these behavioral effects are uncertain, but probably reflect long-term changes in the functioning of 5-HT or DA systems, because MDMA acts as an agonist at 5-HT and DA synapses.

Hypotheses

In adult animals, repeated exposure to MDMA causes 5-HT neurotoxicity. In particular, MDMA induces a massive release of 5-HT, resulting in depleted levels of 5-HT, reduced concentrations of 5-HIAA, decreased activity of TPH, and neurodegeneration of 5-HT axon terminals. In addition to altering 5-HT systems, MDMA, to a lesser extent, impacts DA systems. The purpose of this thesis, therefore, was to determine whether exposing rats to MDMA during the preweanling period causes long-term measurable changes on various indices of 5-HT and DA functioning. The preweanling rat was chosen as the animal model because Broening et al. (2001) have recently shown that early postnatal MDMA exposure causes long-term learning and memory deficits. It was hypothesized that rats exposed to
repeated high doses of MDMA would show a decreased levels of 5-HT, 5-HIAA, DA, DOPAC and reduced PKA activity in dorsal striatum, prefrontal cortex, and hippocampus. These neuronal changes would be consistent with the hypothesis that repeated MDMA exposure during the preweanling period causes long-term damage to 5-HT and DA neurons in rat brain.
CHAPTER TWO
METHODS AND MATERIALS

Animals and Rearing Conditions

Nulliparous female (151-175 g) Sprague-Dawley CD rats were obtained from Charles River Laboratories (Raleigh, NC). Rats were allowed to acclimate to housing conditions in the vivarium at the Cincinnati Children’s Hospital Research Foundation for at least two weeks prior to breeding. Rats were pair housed in polycarbonate cages with lights on from 6:00 to 20:00. Food and water were freely available. On the day of breeding, one female was placed with one male Sprague-Dawley CD rat in a hanging wire cage until a sperm plug was detected [embryonic day 0 (ED 0)], or until the female had been with the male for 2 weeks. Two weeks after being placed with the male, the females were transferred back to polycarbonate cages and singly housed. The presence of a litter was checked twice daily starting on ED 21 and the day of birth was considered postnatal day 0 (PD 0). Litters remained undisturbed until PD 1, at which time litters were culled to ten pups (six males and four females). Subjects were treated according to the National Institute of Health guidelines for the care and use of laboratory animals.
(Principles of Laboratory Animal Care, NIH Publication #85-23) under research protocols approved by the Institutional Animal Care and Use Committees of the Cincinnati Children's Hospital Research Foundation and California State University, San Bernardino.

In Vivo MDMA Administration

Each animal in the litter was randomly assigned to receive either MDMA or saline (n = 7 males and females per group). MDMA HCl (20 mg/kg, expressed as the freebase) or saline vehicle were administered twice daily from PD 11-20. MDMA and saline were injected subcutaneously in the back at a volume of 3 ml/kg. Injection sites were rotated to ensure the surrounding dermis did not become aggravated. Animals were weighed prior to each injection and thereafter at weekly intervals. On approximately PD 60 the animals were shipped to the vivarium at California State University, San Bernardino. Rats were left undisturbed until PD 90, when they were killed by rapid decapitation. Brains were quickly removed and dorsal striatal (i.e., caudate-putamen), prefrontal cortex, and hippocampus were dissected on dry ice and stored at -80°C until time of assay.
Protein Kinase A (PKA) Assay

PKA assays were performed using the Protein Kinase A (cAMP-dependent protein kinase) Assay System protocol (Life Technologies, Grand Island, NY) with slight modifications. Briefly, frozen striatal and prefrontal cortex sections were placed in homogenization buffer [50 mM Tris (pH 7.4), 100 ng/ml leupeptin, 100 ng/ml aprotinin, and 5 mM EDTA] and homogenized using a hand-held Teflon homogenizer. Protein concentrations were determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA) based on the method of Bradford (1976), using bovine serum albumin (BSA) as a standard.

Duplicate striatal homogenates containing 4 µg of protein for each subject were incubated for 5 min at 30°C in phosphorylation buffer [50 mM Tris (pH 7.4), 10 mM MgCl$_2$, and 0.25 mg/ml BSA], containing 50 µg of kemptide and 100 µM [γ-$^{32}$P]ATP (ICN, Costa Mesa, CA). In addition, the buffer contained either cAMP (10 µM) or PKI(6–22) amide (1 µM/reaction). Following incubation, the phosphorylation mixture was blotted on phosphocellulose units (SpinZyme Format). Phosphocellulose units were centrifuged at 20,000 $\times$ $g$ for 30 s, upon completion 500 ml
wash solution and 75 mM phosphoric acid were added and the samples were centrifuged again. Buckets were then transferred into a new receptacle and 500 ml of wash solution was added and centrifuged at 20,000 × g for 30 s. Filters were then placed in scintillation fluid and quantified by liquid scintillation spectrometry. cAMP-dependent PKA activity was defined as the difference between PKA activity in the presence of cAMP and that measured in the presence of PKI.

Serotonin (5-HT) and 5-Hydroxyindoleacetic Acid (5-HIAA) Content Assays

Frozen striatal, prefrontal cortex, and hippocampal sections were sonicated in 10 volumes of 0.1 N HClO₄ and centrifuged at 20,000 × g for 30 min at 4°C. The supernatant was then filtered through a 0.22 µm centrifugation unit at 2,000 × g for 5 min at 4°C. The remaining pellet from each tissue sample was resuspended in 0.1 N HClO₄ and used for protein determination. Twenty microliters of the resulting extracts were then assayed for 5-HT and 5-HIAA using high performance liquid chromatography (582 pump and an MD-150 column; ESA, Chelmsford, MA) with electrochemical detection (Coulochem II; ESA). The mobile phase consisted of 75 mM NaH₂PO₄, 1.4 mM 1-octane sulfonic acid, 10 mM EDTA, and 10%
Dopamine (DA) and Dihydroxyphenyleacetic Acid (DOPAC) Content Assays

Frozen striatal, prefrontal cortex, and hippocampal sections were sonicated in 10 volumes of 0.1 N HClO₄ and centrifuged at 20,000 × g for 30 min at 4°C. The supernatant was then filtered through a 0.22 µm centrifugation unit at 2,000 × g for 5 min at 4°C. The remaining pellet from each tissue sample was resuspended in 0.1 N HClO₄ and used for protein determination. Twenty microliters of the resulting extracts were then assayed for DA and DOPAC using high performance liquid chromatography (582 pump and an MD-150 column; ESA, Chelmsford, MA) with electrochemical detection (Coulochem II; ESA). The mobile phase consisted of 75 mM NaH₂PO₄, 1.4 mM 1-octane sulfonic acid, 10 mM EDTA, and 10% acetonitrile [MD-TM Mobile Phase (pH 3.1); ESA] and was pumped at a rate of 0.5 ml/min.

Data Analysis

Analyses of variance (ANOVA) were used to analyze PKA, DA, DOPAC, 5-HT, and 5-HIAA data. Litter effects were controlled by treating litter as a random factor in all
analyses (see Hughes, 1979). In all cases, alpha were set at 0.05.
CHAPTER THREE

RESULTS

Effects of MDMA on Serotonergic Functioning

Rats pre-exposed to MDMA during the preweanling period had significantly lower 5-HT levels in the prefrontal cortex than saline controls (see upper graph, Figure 1) [drug main effect, $F(1,18) = 12.22, p < .01$]. 5-HIAA levels in the prefrontal cortex also appeared to be depressed relative to saline controls, but the differences between the saline- and MDMA-pre-exposure groups did not reach statistical significance (see lower graph, Figure 1).

Overall, when compared to saline controls, MDMA significantly lowered 5-HT levels in the hippocampus (see upper graph, Figure 2) [drug main effect, $F(1,18) = 87.06, p < .01$]. In addition, male rats had lower levels of 5-HIAA than females [sex main effect, $F(1,18) = 7.53, p < .05$]. MDMA pre-exposure did not affect 5-HT or 5-HIAA levels in the striatum (see Figure 3).

Effects of MDMA on Dopaminergic Functioning

Examination of the prefrontal cortex revealed significantly lower DA levels in rats pre-exposed to MDMA.
during the preweanling period (see upper graph, Figure 4) [drug main effect, $F(1,18) = 5.83, p < .05$]. Unexpectedly, DOPAC levels varied according to sex, since male rats displayed significantly lower DOPAC levels in the prefrontal cortex than females (see lower graph, Figure 4) [sex main effect, $F(1,18) = 4.70, p < .05$].

DA levels in the dorsal striatum varied according to both sex and drug, as only male rats pre-exposed to MDMA showed a significant decline in striatal DA levels (see upper graph, Figure 5) [drug x sex interaction, $F(1,18) = 4.79, p < .05$]. DOPAC levels in the dorsal striatum did not vary according to group (see lower graph, Figure 5). DA and DOPAC levels in the hippocampus were nearly undetectable, so results were not reported.

Effects of MDMA on PKA Activity

Rats pre-exposed to MDMA during the preweanling period showed a significant decline in PKA activity in the prefrontal cortex (see Figure 6) [drug main effect, $F(1,18) = 10.85, p < .05$]. Examination of the hippocampus also revealed that MDMA exposure caused a significant reduction in PKA activity (see Figure 7) [drug main effect, $F(1,18) = 6.66, p < .05$]. MDMA-exposure did not significantly affect dorsal striatal PKA activity,
although female rats had greater PKA activity in the striatum then male rats [see Figure 8] (drug main effect, $F(1,18) = 5.33, p < .05$).

Figure 1. Mean (±S.E.M.) 5-HT and 5-HIAA Levels of MDMA- and Saline-Pre-Exposed Male and Female Rats in Prefrontal Cortex
Figure 2. Mean (±S.E.M.) 5-HT and 5-HIAA levels of MDMA- and Saline-Pre-Exposed Male and Female Rats in Hippocampus
Figure 3. Mean (±S.E.M.) DA and DOPAC Levels of MDMA- and Saline-Pre-Exposed Male and Female Rats in Dorsal Striatum
Figure 4. Mean (±S.E.M.) DA and DOPAC Levels of MDMA- and Saline-Pre-Exposed Male and Female Rats in Prefrontal Cortex
Figure 5. Mean (±S.E.M.) DA and DOPAC Levels of MDMA- and Saline-Pre-Exposed Male and Female Rats in Striatum
Figure 6. Mean (±S.E.M.) PKA Activity Levels of MDMA- and Saline-Pre-Exposed Male and Female Rats in Prefrontal Cortex
Figure 7. Mean (±S.E.M.) PKA Activity Levels of MDMA- and Saline-Pre-Exposed Male and Female Rats in Hippocampus
Figure 8. Mean (±S.E.M.) PKA Activity Levels of MDMA- and Saline-Pre-Exposed Male and Female Rats in Dorsal Striatum
CHAPTER FOUR
DISCUSSION

Rationale

Convincing evidence now exists that MDMA exposure induces both acute and chronic behavioral and neurochemical changes (Bhattacharya et al., 1998; Commins et al., 1987; Marston et al., 1999; Morgan, 1999; Nash & Nichols, 1991; O'Shea et al., 1998). Although the exact method by which MDMA induces neurotoxicity remains to be elucidated, systemic administration of MDMA appears to cause selective and pronounced alterations in both central serotonergic and dopaminergic functioning (Battaglia et al., 1988; Hotchkiss & Gibb, 1980; Insel et al., 1989; Ricaurte et al., 1988). Even though there is sufficient data to indicate that MDMA produces neurochemical alterations in adults, little is known about the possible adverse effects of MDMA exposure on early brain development and functioning. Thus, the purpose of the present study was to determine whether exposure to MDMA during the preweanling period produced long-term changes in various indices of 5-HT and DA functioning.

To that end, male and female rats were pre-exposed to MDMA (20 mg/kg, twice daily) on PD 11-20 and then
sacrificed on PD 90. Neurotoxic damage to 5-HT and DA terminals was determined by measuring decreases in 5-HT, 5-HIAA, DA, and DOPAC levels. Because repeated early treatment with AMPH and METH causes long-term changes in PKA activity (Crawford et al., 2000a, 2000b, 2003), we also examined whether pre-exposure to MDMA would cause a long-term decline in PKA activity in various brain regions.

Evidence for MDMA-Induced Neurotoxicity

Serotonergic and Dopaminergic Functioning

Overall, results from the present study indicate that MDMA exposure during the preweanling period caused neurotoxicity in various brain regions. For example, chronic MDMA exposure produced a significant decrease in 5-HT and DA levels in prefrontal cortex (the metabolites did not differ). Early exposure to MDMA also caused long-term reductions in hippocampal 5-HT levels, suggesting that MDMA was responsible for hippocampal neurotoxicity. The dorsal striatum also evidenced some neurotoxicity, as DA levels, but not 5-HT levels, were depressed in the MDMA group. The latter result was surprising, because both MDMA and METH cause robust 5-HT reductions in adult rat and monkey striatum (De Souza,

In the present study, MDMA-induced neurotoxicity was indirectly inferred by measuring regional reductions in 5-HT and DA levels. There is a substantial literature showing that prolonged reductions in neurotransmitter levels reflects cellular neurotoxicity (for reviews, see Commins et al., 1987; Ricaurte et al., 1985; Schmidt et al., 1986; Stone et al., 1986). For example, decreases in 5-HT and DA concentrations are associated with a loss of functional 5-HT and DA uptake sites, indicating damage to both the serotonergic and dopaminergic nerve terminals (Schmidt, 1987; Schmidt et al., 1987). Furthermore, Ricaurte et al. (1985) found morphological evidence of nerve degeneration in brain regions corresponding to those in which drug treatments had produced selective long-lasting reductions in serotonergic neuronal markers. In addition, histological evidence also revealed degenerating neuronal cell bodies throughout various brain regions having reduced 5-HT and DA levels (Commins et al., 1987; Ricaurte et al., 1985; Schmidt et al., 1986). Thus, analyzing 5-HT and DA levels, although an
indirect method for quantifying neuronal damage, is a valid and recognized measure of neurotoxicity.

PKA Activity

PKA is a central component of the cAMP second messenger system (Taylor et al., 1990). A number of studies have suggested that PKA activity is intimately involved in the mediation of various behaviors, such as locomotor activity, memory, reward-related learning, and anxiety (Abel, Nguyen, Barad, Deuel, Kandel, & Bourtchouladze, 1997; Beninger & Miller, 1998; Micheau & Riedel, 1999; Schafe & LeDoux, 2000; Tolliver, 1999). This is of particular interest, because repeated psychostimulant treatment alters PKA activity (Crawford et al., 2000a; 2000b, 2003; Nestler & Aghajanian, 1997). More specifically, recent studies have described long-term reductions in striatal and accumbal PKA activity following AMPH (2.5-5.0 mg/kg/day) and METH (40 mg/kg/day) exposure during the preweanling period; thus, it is possible that drug-induced changes in PKA activity may have substantial long-term behavioral impact (Crawford et al., 2000a, 2003).

To date, no studies have examined the long-term effects of developmental exposure to MDMA on PKA activity. In the present study, rats given MDMA on PD 11-20 showed
reduced levels of PKA activity in the prefrontal cortex and hippocampus. Crawford et al. (2000a, 2000b) have previously postulated that psychostimulant-induced alterations in the DA system (e.g., an up-regulation of D₂-like receptors or a down-regulation of D₁-like receptors) by altering cAMP system functioning may have depressed PKA activity (Crawford et al., 2000a, 2000b). The present results are consistent with this explanation. However, in the present study we found that MDMA caused a robust reduction in prefrontal and hippocampal 5-HT levels. Some of the 5-HT receptor subtypes (i.e., 5-HT₁₅, 5-HT₁₆, 5-HT₁₇, 5-HT₄, 5-HT₆, & 5-HT₇) utilize the cAMP second messenger system (Cooper et al., 1996), so it is possible that alterations in 5-HT could be one of the driving forces (along with DA) responsible for MDMA-induced changes in PKA activity. This possibility is indirectly supported by results showing that those brain areas exhibiting the greatest reductions in 5-HT levels also exhibited the greatest reductions in PKA activity. Although the exact neural mechanisms have not been determined, it is possible that MDMA's ability to reduce neuronal 5-HT and DA levels is responsible for the decline in PKA activity.
Although MDMA’s actions were generally similar in both sexes, it is curious that only male rats showed an MDMA-induced reduction in striatal DA levels. This pattern of results has been reported before, since male rats are often found to be more susceptible to psychostimulant-induced neurotoxicity (for a review, see Dluzen, 2000). Past research has shown that circulating estrogen reduces the neurotoxic effects of AMPH and METH (Gao & Dluzen 2001; Wagner, Tekirian, & Cheo, 1993), and the present results are consistent with the hypothesis that the neuroprotective effects of estrogen may extend to MDMA. Additional sex differences were also apparent, as male rats had lower levels of hippocampal 5-HIAA and prefrontal DOPAC than female rats. The importance of the latter findings is uncertain.

**Summation**

As mentioned earlier, MDMA reduces TPH activity, 5-HT transporter sites, and causes degeneration of 5-HT axon terminals in adult animals (Battaglia et al., 1987, 1988; O’Shea et al., 1998). MDMA has also been shown to affect DA functioning in adults. More specifically, MDMA alters the number of DA transporters, and reduces both TH activity and DA levels in adult animals (Hotchkiss & Gibb,
1980; Metzer et al., 1998; Nash & Nichols, 1991; Wagner et al., 1980).

Few studies have examined whether fetal brain damage occurs when pregnant dams are exposed to MDMA. However, available evidence suggests that MDMA exposure during gestation has little effect on postnatal neurochemical development (St. Omer et al., 1991). It has also been reported that preweanling rats pre-exposed to MDMA on PD 1-10 do not exhibit neuronal or behavioral deficits (Broening et al., 2001; St. Omer et al., 1991). Only recently has it become clear that exposing rats to MDMA on PD 11-20 causes long-term deficits in cognitive functioning (Broening et al., 2001). Importantly, the late preweanling period in rats (i.e., PD 11-20) is roughly analogous to the late 3rd trimester of human brain development (Bayer, Altman, Russo, & Zhang, 1993). Thus, the present study has provided new evidence suggesting that MDMA exposure can cause neurotoxic effects at doses that approach or mimic those used by recreational MDMA users (Ricaurte & McCann, 1992). Of special concern are pregnant MDMA users who expose their unborn fetus to MDMA and, in this way, increase the risk of neurological damage at developmental brain stages.
More generally, psychological disorders and psychiatric symptoms, such as panic attacks (Whitaker-Azmitia & Anderson, 1989), depression (Cohen, 1996), and suicidal ideation (Benazzi & Mazzoli, 1991) have been associated with MDMA use. The question continues to surface whether there is a causal link between MDMA use and the development of psychiatric disorders or whether MDMA exacerbates a neurological condition among individuals predisposed (Spitzer, Franke, Walter, Buechler, Wunderlich, Schwab, Kovar, Hermle, & Gron, 2001). If MDMA is neurotoxic in humans, as has been shown in other species, then it is likely that the wide-spread use of MDMA will present a major public health problem in years to come.

Conclusion

The present study has demonstrated that repeated exposure to MDMA produces long-term damage to serotonergic and dopaminergic neurons in various regions of rat brain. This conclusion is supported by data showing that MDMA pre-exposure: (a) decreased 5-HT levels in prefrontal cortex and hippocampus; (b) decreased DA levels in prefrontal cortex and dorsal striatum; and (c) reduced PKA activity in prefrontal cortex and hippocampus. Other
investigators have examined the effects of early MDMA-exposure on a variety of behavioral and cognitive paradigms. For example, data indicate that MDMA exposure during the late preweanling period causes significant disruptions in sequential and spatial reference memory-based learning (Broening et al., 2001). These behavioral deficits could potentially be accounted for by MDMA-induced reductions in 5-HT and DA levels, as well as by changes in PKA activity. Thus, our findings not only increase our knowledge of the neurochemical changes produced by early MDMA exposure, they also provide insight into human brain neurotoxicity and those MDMA-induced changes that may be responsible for learning and memory deficits and psychiatric disorders.
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