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# SHORT AND LONG-TERM EFFECTS OF MDMA EXPOSURE IN RODENTS: PHYSIOLOGICAL, BEHAVIORAL AND NEUROCHEMICAL RESPONSES

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**by**

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**Dissertation**

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

**Doctor of Philosophy**

**The University of Texas at Austin**

**May 2006**

# Dedication

This dissertation is dedicated to my parents “Angeles” and “Luis” and to my husband “Carlos”, without their encouragement, and support this dream would not have been possible. Their love and pride of my research have been an inspiration for me to complete this work.

# Acknowledgements

First, I would like to acknowledge and thank my advisor Christine L. Duvauchelle for her guidance, trust and unconditional support. I will also like to express my sincere gratitude to my previous advisors Gary W. Miller and Terrence J. Monks. I also acknowledge the help and support of the other members of my committee. Last but not least, to my lab mates over the years that cheered me up and helped when I needed it.

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Publication No._____

Maria Elena Reveron, Ph.D.

The University of Texas at Austin, 2006

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3,4-methylenedioxymethamphetamine (MDMA) is a popular abused amphetamine among young adults. The possibility that MDMA intake may be neurotoxic in humans led to a wealth of studies that demonstrated large doses of MDMA cause damage to serononin (5-HT) nerve terminals in rats and non-human primates. However, to date, the exact mechanism of MDMA-induced neurotoxicity in animals is unknown. In the first data set of this dissertation, neurochemical and thermal changes associated with experimenter-delivered repetitive doses of MDMA in mice of two different age groups were investigated. Confirming previous findings with mice, repetitive MDMA administration affected dopamine (DA) system markers. Findings revealed, significant decreases in vesicular monoamine transporter2 (VMAT2) protein and significant increases in rectal temperature in older mice compared to younger counterparts. The data suggest older mice are more sensitive to the toxic effects of MDMA and that hyperthermia might contribute to MDMA neurotoxicity.

Few studies have examined MDMA effects using drug self-administration procedures. Therefore, the remaining portion of the dissertation involved a series of studies investigating behavioral and biological changes occurring as the result of short- and long-term MDMA self-administration. MDMA-stimulated lever responses, locomotor activity, thermal effects, *in vivo* changes in nucleus accumbens (NAcc) DA and 5-HT levels and post-mortem tissue content of DA, and 5-HT were determined after various periods of MDMA abstinence in MDMA-naïve animals, and those self-administering over 20 daily sessions. In general, experience-dependent changes in MDMA were observed in all assessed measurements. For instance, MDMA-stimulated locomotor activation increased with experience, initial hypothermia induced by MDMA progressively reversed over time, proportional changes in NAcc DA and 5-HT were altered with experience, and depleted tissue levels of 5-HT recovered after MDMA abstinence. An additional study revealed that many of these experience-dependent changes might be mediated through actions at the 5-$HT_{2c}$ receptor. For instance, in animals with a single MDMA experience, the 5-$HT_{2c}$ antagonist SB242084 enhanced MDMA-induced NAcc DA levels and locomotor activity in a manner similar to that observed only in the long-term MDMA experienced rats. In conclusion, these results suggest MDMA intake induces physiological, neurochemical and behavioral responses that change with increased MDMA drug experience.

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# GENERAL INTRODUCTION

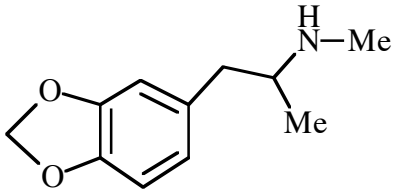
When Merck Pharmaceuticals first synthesized the drug 3,4-methylenedioxymethamphetamine (MDMA) in 1914 (Merk, 1914), it was conceived as an appetite suppressant. However, it was Alexander Shulgin, a chemist and "the godfather of Ecstasy", who made the drug available to the general public and defended its use as an adjuvant in psychotherapy (Shulgin, 1995). In 1985, the U.S. government placed this drug in the Schedule I category of psychoactive controlled substances (www.usdoj.gov/dea). Today MDMA is a popular illegal drug among teenagers, and is commonly known on the street as Ecstasy, Adam, Doves, XTC and Cadillac, among others. It is normally ingested orally, primarily at dance clubs (Parrott, 2002). Users often employ adjunct sensory stimuli when using MDMA, such as fluorenscent light sticks, which they wave rapidly in front of their eyes to increase visual stimulation and over-the-counter products, such as Vicks Vapor Rub, to enhance olfactory stimulation. Recent research indicates MDMA use has spread beyond nightclubs to places such as private homes and high schools (Bialer, 2002). Ecstasy pills are sold in a variety of colors and are imprinted with various brand logos (e.g. Mitsubishi, Rolls Royce, Channel). Ecstasy pills contain the racemic mixture of MDMA. Numerous works have demonstrated each isomer has unique neuropharmacologic and psychostimulant properties (Baker et al., 1997; Glennon et al., 1988; Johnson et al., 1988; Lyon et al., 1986; McKenna et al., 1991; Rosecrans and Glennon, 1987). Pills have been reported to contain between 60-100 mg of MDMA (Bialer, 2002; Wolff et al., 1995; Cole et al., 2005). MDMA effects begin 20-120 min after its ingestion, peaks after 2 hours, and last for about 3 hours (Liechti, 2000). Tablets oftentimes are combined with cheaper compounds such as caffeine, ephedra,

dextromethorphan, amphetamine, methamphetamine, or MDMA derivatives (Baggott et al., 2000).

Typically human recreational doses are on the order of 1-2 mg/kg (Hegadoren et al., 1999; Peroutka, 1987). Users say they take Ecstasy because it increases their self-confidence, peacefulness, and feelings of pleasure and emotional closeness (Cohen, 1998; Vollenweider et al., 1998; Yamamoto and Spanos, 1988).

MDMA: CHEMICAL STRUCTURE

MDMA is an amphetamine derivative with both stimulant and hallucinogenic properties. Its chemical molecule consists of a benzene ring, an N-methyl-a-methyl-ethylamine side chain and a methylenedioxy group joining the 3,4 phenyl carbons to form a ring. MDMA has two optical isomers: R(-) and S(+).




Its structure resembles that of several neurotransmitters in the brain, such as dopamine (DA) and serotonin (5-HT). Hence, MDMA acts as a false neurotransmitter at the nerve terminal, promoting the massive release of authentic neurotransmitters (5-HT, DA, norephinephrine, and acetylcholine) from their presynaptic terminals (Acquas et al., 2001; Callaway et al., 1990; Fischer et al., 2000; Fitzgerald and Reid, 1990; Gough et al.,

1991; Gudelsky and Nash, 1996; McKenna et al., 1991; Nichols et al., 1982; Yamamoto et al., 1995). At the same time, MDMA blocks the re-uptake of these neurotransmitters potentiating the sympathetic nervous system activity (Hekmatpanah and Peroutka, 1990; Johnson et al., 1986; Nichols et al., 1982; Rudnick and Wall, 1992).

## MDMA: NEUROTOXICITY RESEARCH

There is public concern regarding the wide spread use of MDMA among young people since little is known about the potential hazardous effects of this drug (Itzhak and Achat-Mendes, 2004; Kish, 2002; Pham and Puzantian, 2001). For the last two decades, MDMA research had concentrated on the possible toxic effect of MDMA use on brain 5-HT cells. Neurotoxicologists investigate the adverse effects of drugs with the help of *in vitro*, and experimental animals. Interspecies extrapolation is used to validate the results obtained from animal research to human populations (Winneke and Lilienthal, 1992). Accordingly, the amount of MDMA used in these experiments is greatly in excess to that used by humans. Most studies on MDMA neurotoxicity have been conducted in rodents where prolonged, or very high doses of the drug, have been tested.

## ASSESSMENT AND POTENTIAL MECHANISMS OF MDMA-INDUCED NEUROTOXICITY: ANIMAL STUDIES

Early reports (Schmidt et al., 1987; Stone et al., 1986) showed that either acute high doses, or chronic MDMA exposure caused a marked depletion in 5-HT content in rats. Apparently, MDMA-induced 5-HT loss follows a biphasic pattern that starts with an acute depletion observed as soon as 3 hours after MDMA exposure, which recovers after

24 hours, and an irreversible decrease a week later that persists for up to one year. Several brain regions of the rat forebrain are affected following MDMA administration; the neocortex and hippocampus among the most severely damaged (Battaglia et al., 1987). In addition, long-term decreases in striatal 5-HT levels are also observed (Battaglia et al., 1988; Stone et al., 1986). Other markers of serotonergic integrity are affected upon MDMA exposure. Levels of 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of 5-HT are reduced after a single, or repetitive doses of MDMA (Schmidt et al., 1987; Schmidt et al., 1986; Stone et al., 1987b). The activity of tryptophan hydroxylase (TPH), the rate-limiting enzyme in 5-HT biosynthesis, is also reduced when MDMA is administered acutely to rats (Schmidt and Taylor, 1987; Stone et al., 1987b). After an initial rise, 5-HT synthesis is halved 5 hours post-MDMA (Nishisawa et al., 1999). MDMA decreases TPH activity in a concentration-dependent manner, and different hypotheses have been proposed to explain the mechanism involved. Possibilities include oxidation of sulphydryl residues by peroxynitrite (Kuhn and Geddes, 1999; Schmidt and Taylor, 1987), oxidation of thiol groups in the enzyme (Stone et al., 1989), or feed-back control upon binding of extracellular 5-HT to autoreceptors (Schmidt et al., 1992).

In the serotonergic system, the 5-HT transporter (SERT) is responsible for the selective uptake of 5-HT from the synaptic cleft into the presynaptic terminal. 5-HT transmission and actions are terminated by this active transport (Bruns et al., 1993; Pan and Williams, 1989). Not surprisingly, SERT is the target of amphetamine derivatives that affect the serotonergic system, including MDMA (Johnson et al., 1986; Nichols et al., 1982; Pletscher and Bartholini, 1967). Additionally, 5-HT selective reuptake inhibitors (SSRIs), such as fluoxetine block SERT, and protect against MDMA-induced

neurotoxicity (Battaglia et al., 1988; Commins et al., 1987). SERT is considered a very reliable index of terminal integrity, and reductions in SERT density observed in animals exposed to serotonergic toxicants correlate to nerve terminal degeneration (Battaglia et al., 1987; Brown and Molliver, 2000). Neuroimaging studies performed in ecstasy users, using radiolabeled ligands that specifically bind to SERT, have demonstrated global reductions in SERT binding (McCann et al., 1998; Ricaurte et al., 2000).

Surprisingly, MDMA does not affect the 5-HT neuron cell body (O'Hearn et al., 1988). The gold standard for assessing neuronal damage is tissue staining. Silver staining and immunohistochemistry have shown serotonergic axons to be fragmented and swollen in rats and monkeys (Battaglia et al., 1987; O'Hearn et al., 1988; Sprague et al., 1998). However, the cell bodies in the raphe nuclei, the area from where most serotonergic axons arise, remain intact. Finally, gliosis (an increase in the number of glial cells) represents indirect evidence of neuronal damage. Increases in the glial fibrillary acidic protein, or GFAP, have also been observed after MDMA administration (Miller and O'Callaghan, 1995).

Stone and colleagues (Stone et al., 1987a) measured neostriatal concentrations of 5-HT, DA, and its metabolites in both rats and mice after a single injection of MDMA. In that study, the authors concluded rats were more sensitive to the toxic effects of MDMA and suggested differences in drug metabolism might prolong the half-life of the drug in rats. Ring para-hydroxylation is the major metabolic pathway in rats, while in mice, side-chain metabolism is equally important (Caldwell, 1976). In rats, the methyldioxy group of MDMA might interfere with the metabolism of this amphetamine derivative. To the contrary, side-chain metabolism in mice might contribute to MDMA clearance.

Toxicology studies have shown the acute LD50 for rats is 49 mg/kg i.p., whereas for mice is 97 mg/kg (Hardman et al., 1973). The existing data indicate MDMA affects rats and mice neurochemistry in a very different way. Steel and coworkers (1991) studied the metabolism of MDMA and found rat liver microsomes produced a significantly greater amount of 3,4-dihydroxymethamphetamine (HHMA), a major MDMA metabolite, than did mice. Whether metabolic differences might account for the difference in neurotoxicity, or not, remains unclear. In addition, while in rats long-lasting changes are observed primarily in the serotonergic system, in mice the dopaminergic system appears to be more severely affected. Subsequent reports have confirmed MDMA elicits significant reductions in DA, DOPAC and HVA, increases of GFAP immunoreactivity and nerve terminal degeneration in the striatum as revealed by silver staining (Colado et al., 2004; Jayanthi et al., 1999; Logan et al., 1988; Miller and O'Callaghan, 1995; O'Callaghan and Miller, 1994; O'Shea et al., 2001).

In the dopaminergic system the plasmalemmal dopamine transporter (DAT) and tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis, are considered reliable biomarkers of axonal integrity. DAT regulates the intensity and duration of synaptic transmition by removing DA from the synapse. The DAT is a member of a large family of $Na^+/Cl^-$-dependent transporters, including the closely related NE transporter. It co-transports DA and $Na^+$ into the synaptic terminal and represents the major mechanism for extracellular neurotransmitter clearance (Giros and Caron, 1993; Giros et al., 1991). It has been shown that pretreatment with a DAT inhibitor (GBR 12920) prevents the long-term MDMA-mediated neurotoxicity in mice (O'Shea et al., 2001).

There are several proposed mechanisms to explain MDMA-induced neurotoxicity, Central administration of MDMA produces the release of neurotransmitters, but fails to cause the long-lasting damage (Esteban et al., 2001; Paris and Cunningham, 1992; Schmidt and Taylor, 1988). For this reason, several investigators have suggested MDMA needs to be metabolized peripherally in order to cause neurodegeneration (Bai et al., 1999; Carvalho et al., 1996; Hiramatsu et al., 1990; Miller et al., 1995).

Extracellular DA levels are increased following MDMA treatment (Schmidt et al., 1986; Steele et al., 1987; Stone et al., 1986). The mechanism underlying MDMA-induced DA release is unclear but might involve both blockage and reversal of DAT, and impulse-mediated exocytosis (Bankson and Cunningham, 2001; Crespi et al., 1997; Metzger et al., 1998; Yamamoto and Spanos, 1988). In addition, evidence suggests there is a correlation between acute DA release and degree of serotonergic damage observed in rats (Nash and Nichols, 1991). These data led researchers to believe DA might play an important role in the mechanism of MDMA-mediated serotonergic axonal loss (Stone et al., 1988).

## OXIDATIVE STRESS

There is strong evidence that oxidative stress is involved in the mechanism of MDMA neurotoxicity (Cadet et al., 2001; Camarero et al., 2002; Colado et al., 1997; Sanchez et al., 2003; Zhou et al., 2003). Antioxidants such as ascorbate reduce neurotoxicity in rats without altering the amount of MDMA, or dopamine release (Gudelsky, 1996). The development of various transgenic knockout mouse models has

helped elucidate various mechanisms underlying MDMA-mediated neurotoxicity. Homozygous and heterozygous copper/zinc superoxide dismutase (an antioxidant enzyme) transgenic mice were partially protected against MDMA-mediated DA damage (Jayanthi et al., 1999). Moreover, MDMA causes a decrease in catalase and glutathione peroxidase, and an increase in lipid peroxidation in wild-type animals, effects that are not observed in the homozygous mice (Jayanthi et al., 1999). Oxidative stress might be caused by metabolites of either DA, 5-HT, and/or MDMA. Evidence suggests enhanced DA neurotransmission plays a major role in MDMA-induced serotonergic toxicity (Schmidt et al., 1990b; Stone et al., 1988). As previously mentioned, *in vivo* microdialysis techniques demonstrate MDMA administration induces dopamine release in various brain regions (Colado et al., 1999; Gough et al., 1991; Gudelsky et al., 1994; Nash and Brodkin, 1991; Yamamoto and Spanos, 1988). Several hypotheses, including excess extracellular DA taken up by 5-HT presynaptic terminals (Sprague et al., 1998), have been proposed.

## EFFECTS OF MDMA ON BODY TEMPERATURE

It is a well-known fact MDMA interferes with thermoregulation in humans and animals. Most studies, conducted at room temperature 21°C or higher, reveal administration of large doses of MDMA induce hyperthermia, with higher rectal temperatures reported at warmer environments (Broening et al., 1995; Dafters, 1994; Malberg et al., 1996; Nash et al., 1988; O'Shea et al., 1998). This particular feature raises some concern, since ecstasy abusers ingest the drug mostly in closed environments, such as dance clubs, and all-night vigorously dancing aggravates the marked increased in core temperature. In addition, pharmacological studies have demonstrated MDMA-mediated

increase in body core temperature is dose-dependent (Carvalho et al., 2002; Fantegrossi et al., 2003; Malberg and Seiden, 1998; Nash et al., 1988; O'Shea et al., 1998).

The exact mechanism responsible for MDMA-associated hyperthermia in humans remains unknown. The process of thermoregulation in mammals occurs through autonomic and behavioral responses. The hypothalamus is believed to be the central thermostat that regulates certain body functions including body temperature (Satinoff, 1978; Simon, 1974). In rats, MDMA induces an increase in the metabolic rate and a reduction of the blood flow to the tail (Gordon and Fogelson, 1994), and might interfere with mechanisms that mediate heat loss (e.g. vasodilation of tail vessels; Mechan et al., 2002). There has been some speculation on whether DA or 5-HT mediate these MDMA-induced changes in body temperature (Mechan et al., 2002; Shankaran and Gudelsky, 1999).

Hyperthermia is a well-known pro-oxidant aggressive condition. Therefore, MDMA-induced increases in core temperature might partially be responsible for its neurotoxic effects. In rats, prevention of the hyperthermic responses achieved by cold ambient temperatures/restrain stress conditions has been shown to be neuroprotective (Malberg and Seiden, 1998; Miller and O'Callaghan, 1994; Miller and O'Callaghan, 1995). Likewise, pharmacological treatments (e.g. dizocilpine (a N-methyl-D-aspartate receptor antagonist), alpha-methyl-p-tyrosine (catecholamine synthesis inhibitor)) that reduce core temperature attenuate the toxicity of MDMA (Farfel and Seiden, 1995; Malberg et al., 1996). However, there is evidence showing pharmacological protection against MDMA-induced serotonergic neurotoxicity is not dependent on changes in body temperature (Malberg et al., 1996; Schmidt et al., 1990a). For example, pretreatment of

mice with fluoxetine inhibited the observed changes in body temperature but had no effect on MDMA-associated DA damage (O'Shea et al., 2001). Therefore, the nature of the relationship between MDMA-mediated neurotoxicity and increased body temperature is still unclear.

## MDMA: EFFECTS OF LOW-MODERATE DOSAGES

Much of the scientific literature on MDMA focus on issues related to the potential of MDMA to induce neurotoxic effects, such as long-lasting depletion of 5-HT, 5-HIAA or decrease in TPH activity. However, additional work has been conducted to study the psychopharmacology behind MDMA abuse. In these studies, researchers investigate the short and long-term neurochemical changes associated with low doses, or voluntary MDMA administration. In addition, a few studies have investigated the molecular mechanisms underlying MDMA rewarding properties. For example, Fantegrossi and collegues (Fantegrossi et al., 2004) investigated the effect of chronic MDMA self-administration (18 months) in rhesus monkeys on various 5-HT system markers. The authors concluded chronic MDMA self-administration was not accompanied by long-lasting changes in 5-HT biochemistry in these animals. Recently, researchers have started to investigate the physiological and behavioral changes associated with single or repetitive small doses of MDMA, to closer resemble the human recreational dose regimen (Baker and Makhay, 1996; Cadoni et al., 2005; Green et al., 2004; Sanchez et al., 2004).

## HUMAN PSYCHOLOGICAL EFFECTS

MDMA has been pharmacologically classified as a new category of drug called "entactogen" from the Latin "to touch within" (Nichols, 1986). The reason for this is MDMA consumption induces feelings of euphoria, self-confidence, well-being and connectedness with others (Cami et al., 2000; Liechti et al., 2000). Because of MDMA peculiar properties some psychiatrist thought of MDMA as a potent adjuvant to psychotherapy (Naranjo, 1973; Naranjo, 1967; Yensen et al., 1976).

However, long-term MDMA usage induces persistent psychological problems that include anxiety, depression, hostility, and aggression (Morgan, 2000; Parrott, 2002), and might cause memory impairment (Gouzoulis-Mayfrank et al., 2000; Reneman et al., 2000).

## ANIMAL STUDIES: BEHAVIORAL ACTIVITY

MDMA administration elicits the so-called serotonin syndrome in laboratory animals (low body posture, forepaw treading, headweaving, piloerection, proptosis, penile erection, ejaculation, salivation, and defecation) characteristic of increased 5-HT activity (Green et al., 1995; Hiramatsu et al., 1989). Likewise, MDMA causes a dose-dependent increase in locomotor activity in rodents and non-human primates (Callaway et al., 1990; Gold et al., 1988; Slikker et al., 1989), and the 5-HT system might be partially responsible of this effect. The 5-HT uptake blocker fluoxetine inhibited MDMA-mediated hyperactivity (Callaway et al., 1990; Gold et al., 1989) and RU 24969, a 5-$HT_{1B}$ receptor agonist, causes locomotor hyperactivity in rats, whereas agonists for 5-$HT_{1A}$ and 5-$HT_{2A/2C}$ receptors decrease locomotion (Rempel et al., 1993). It has been proposed

increased DA neurotransmission might be partially involved MDMA-mediated increase in locomotion (see diagram page 19). Several *in vivo* studies have demonstrated MDMA is equally effective in releasing DA and 5-HT when injected peripherally (Kankaanpaa et al., 1998; O'Shea et al., 2005; White et al., 1994). To complicate matters further, 5-HT and DA have been shown to influence each other's release by interaction with specific DA or 5-HT receptors within the mesolimbocortical pathway (Matsumoto et al., 1996; Porras et al., 2002). The literature suggests both systems play an important role in MDMA-induced changes in behavior, and therefore, both systems should be studied to better understand the neuropharmacology of MDMA.

Long-term exposure of rats to MDMA induces behavioral sensitization (Kalivas et al., 1998; Modi et al., 2006; Nagilla et al., 1998), a process characterized by enhanced response following repetitive doses of the drug. There is a large body of data suggesting drug dependence might be closely related to the phenomenon of sensitization (Deroche et al., 1999; Robinson and Becker, 1986; Robinson and Berridge, 1993), and increased DA neurotransmission (Di Chiara, 1995; Robinson and Berridge, 1993; Wise and Rompre, 1989). Hence, the study of locomotor sensitization is relevant to understand the mechanisms involved in MDMA reward. Several studies indicate that DA $D_1$ receptors have a primary role in the behavioral sensitization induced by MDMA and other psychostimulants (Bjijou et al., 1996; Henry and White, 1991; Ramos et al., 2004). However, evidence obtained subsequently suggested the involvement of the 5-$HT_{2C}$ receptors as well (Ramos et al., 2004; Scheffel et al., 1992).

## THERMOREGULATION

Preoptic area and anterior hypothalamus thermosensitive neurons play a key role in the regulation of core temperature (Boulant, 1998; Pierau and Schmid, 1990; see diagram page 19). These areas integrate information about local brain temperature as well as temperature from other parts of the body. In rats, heat loss occurs mainly through thermoregulatory tail blood flow. Efferent signals for this response originate in thermosensitive neurons and the descending pathway passes through the medial forebrain bundle. Two midbrain regions seem to participate in this tail vasomotor control: the ventrolateral part of the rostral periaqueductal gray and the ventral tegmental area (VTA). Stimulation of the VTA suppresses vasodilation otherwise elicited by warming of the preoptic area. Blood flow in the rat's tail is control by sympathetic postganglionic fibers. These postganglionic neurons are controlled by preganglionic sympathetic neurons located in the intermediolateral cell column. It is well accepted that sympathetic premotor neurons in the rostral ventrolateral medulla (RVLM) control this area. However, recent evidence suggests the rostral raphe nucleus (RnR) (Morrison, 2001; Tanaka et al., 2002) is more important than the RVLM in controlling the rat's tail flow. A recent study (Mechan et al., 2002) demonstrated that the temperature of the rat tail was unaltered following a dose of MDMA that produced a significant rise in rectal temperature, which suggest MDMA interferes with mechanisms of heat loss in rats. Since MDMA induces a decrease in core temperature when animals are maintained in cool environments, it is possible MDMA might also interfere with mechanism of heat production.

In rats and mice, thermogenesis is mediated by non-shivering mechanisms, and the brown adipose tissue (BAT) is the principal effector (Cannon and Nedergaard, 2004;

Morrison, 2004). Deposits of BAT are distributed throughout the body, but the largest depot is located in the interscapular BAT, and receives sympathetic excitatory cholinergic inputs from preganglionic neurons. These preganglionic neurons receive direct inputs from sympathetic premotor neurons located in the RnR. Mechanisms of thermogenesis involve the activation of uncoupling proteins (UCP) (Mills et al., 2004; Nedergaard et al., 2001), which uncouple oxidative phosphorylation in mitochondria. In humans and experimental rodents, MDMA-induced hyperthermia is mediated by non-shivering mechanisms as well. However, adult humans have little BAT and different tissues (e.g., skeletal muscle) mediate thermogenesis. Evidence suggests MDMA-associated increase in core temperature involves skeletal muscle UCP uncoupling via sympathetic activation (Mills et al., 2003; Mills et al., 2004).

5-HT is the main neurotransmitter involved in thermoregulation, and 5-HT receptors are located centrally and throughout the peripheral nervous and vasomotor systems (Bligh, 1979; Fozard, 1984). Yet, other catecholamines (e.g., DA, NE) are also implicated in these thermoregulatory processes (Preston, 1975; Silva and Larsen, 1983). There is evidence activation of 5-$HT_{1A}$ receptors within the central nervous system reduces sympathetic outflow to BAT, while activation of 5-$HT_{2A}$ receptors increases sympathetic activity and BAT metabolism (Ootsuka and Blessing, 2006). Hence, increase 5-HT release and 5-HT receptor activation in brain regions such as the RnR might be an important mediator of MDMA-associated increase in body temperature. However, NE signaling on sympathetic receptors might also contribute to MDMA-induced hyperthermia (Mills et al., 2004).

MDMA-mediated changes in body temperature are influenced by small changes in ambient temperature (Malberg and Seiden, 1998). At room temperature 22°C or lower,

systemic MDMA administration induce hypothermia (Dafters, 1994; Dafters and Lynch, 1998; Malberg and Seiden, 1998; Piper et al., 2005). In addition, repeated administration of MDMA (7.5mg/kg/day for 13 days) has been shown to induce sensitization of the hyperthermic response (Dafters, 1995).

## REWARD AND REINFORCENT

Reinforcing effects of drugs of abuse are commonly tested using laboratory animal models. With few exceptions, drugs that are abused by humans are also voluntarily self-administered by animals, making animal self-administration models a convenient mean to study the brain mechanisms involved in reward (Bozarth et al., 1980; Gardner, 2000; Koob, 1995; Olds, 1982). In this model, the animals learn to press a lever in an operant chamber to receive an intravenous infusion of the drug through a catheter implanted into the jugular vein. Although MDMA is not as potent a reinforcer compared to cocaine, or methamphetamine, studies have shown MDMA is reliably self-administered by rodents and non-human primates (Beardsley et al., 1986; Fantegrossi et al., 2002; Lamb and Griffiths, 1987; Marona-Lewicka et al., 1996; Ratzenboeck et al., 2001; Schenk et al., 2003; Trigo et al., 2006).

The midbrain region contains two areas that are very important in drug reinforcement: the ventral tegmental area (VTA) and the nucleus accumbens (NAcc). The VTA is rich in dopamine cell bodies that project to the NAcc, and form the mesolimbic dopamine system (see diagram page 19). The NAcc is compartmentally composed of two main subregions: the core (the portion that surrounds the anterior commissure) and the shell (medial-ventral) region. Psychostimulants, such as MDMA, cocaine, or

amphetamine cause their behavioral effect via increased DA neurotrasmission in specific brain regions including the mesolimbic system (Ritz and Kuhar, 1993; Wise, 1980; Yamamoto and Spanos, 1988). Closely associated with this region is the mesocortical dopamine pathway, which projects from the VTA to the prefrontal cortex. The mesocorticolimbic system is a major component of the "reward" system and is associated with drugs of abuse incentive motivational processes. DA and 5-HT release in the NAcc play a central role in the reinforcing properties of drugs of abuse (Di Chiara, 1999; Di Chiara and Imperato, 1988) and both neurotransmitters are increased with MDMA administration. Although the DA system has long been associated with the reinforcing properties of drugs of abuse, few studies have examined the potential contributions of 5-HT to MDMA rewarding effect (Bilsky and Reid, 1991; Ratzenboeck et al., 2001).

MDMA rewarding effects have been studied using various animal models of drug reinforcement. To assess the motivational properties of abused drugs, such as MDMA, researchers utilized various learning paradigms. In the drug self-administration paradigm, the animal presses a lever and receives an intravenous infusion of the drug through an implanted jugular catheter (De La Garza et al., 2006). Reliably responding on the lever is evidence of reinforcing properties of the drug. Several studies have reported MDMA is reliably self-admistered by rodents and non-human primates (Beardsley et al., 1986; Fantegrossi et al., 2002; Ratzenboeck et al., 2001; Schenk et al., 2003). Another popular experimental method for studying drug reward is the conditioned place preference paradigm (Rossi and Reid, 1976). In this model, the drug of choice is repeatedly paired with a distinctive environment. Subsequently, animals are tested in a non-drug state and the preference or aversion to the drug is inferred by the amount of time the animal spends in the drug-paired environment. The rewarding properties of MDMA have been also

demonstrated using this model (Bilsky et al., 1991; Marona-Lewicka et al., 1996; Robledo et al., 2004; Schechter, 1991). Another technique used to assess the positive reinforcing properties of drugs utilizes intracranial self-stimulation (Bozarth et al., 1980; Routtenberg, 1972). This model uses electrical self-administered stimulation of areas of the brain involved in drug reward. It is widely accepted drugs of abuse, including MDMA, lower the threshold of intracranial self-stimulation (Esposito et al., 1979; Hubner et al., 1988; Lin et al., 1997; Kornetsky et al., 1979).

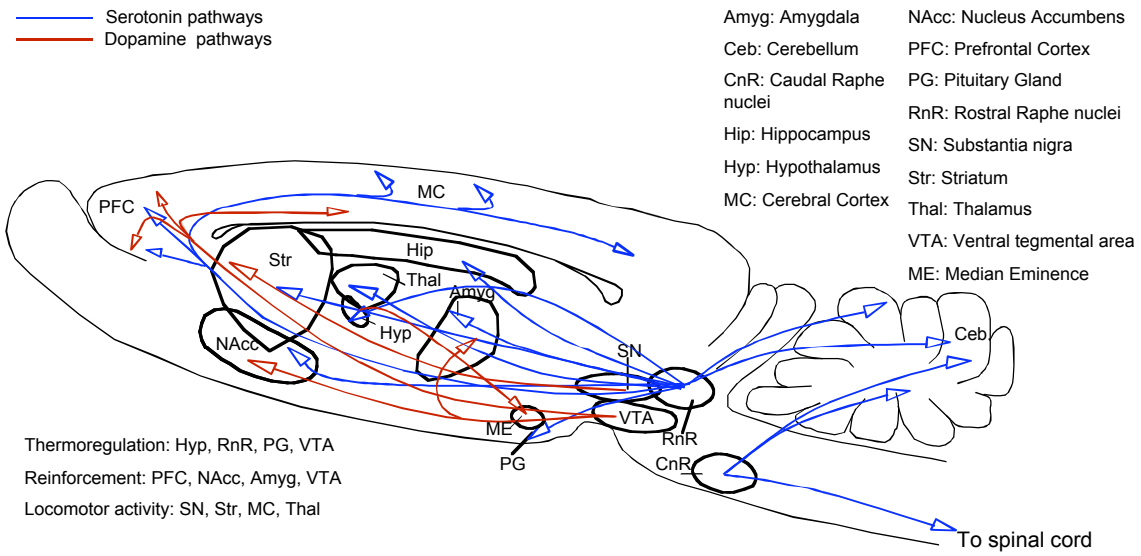
**Goals of this dissertation**

While it shares similarities with other amphetamine derivatives, both experimental and clinical studies demonstrate MDMA has a unique neuropharmacology that results from the interaction between the DA and 5-HT systems. There has been an extensive characterization of MDMA-mediated toxic effects in rats and monkeys in the literature. However, relatively few reports have been published assessing the neurotoxic effects of MDMA in mice. One of the goals of this project is to test the hypothesis that the long-lasting changes observed in the dopaminergic system in mice, after MDMA exposure, are age-dependent and might be influenced by dissimilar effect of body temperature.

Few studies have investigated the rewarding effects of MDMA; though there are many differences between MDMA and other drugs of abuse, such as cocaine, amphetamine, and heroin. Therefore, many questions remain unanswered regarding the behavioral, neurochemical, and physiological changes associated with MDMA self-administration.

With the use of MDMA self-administration and *in vivo* microdialysis, we will increase the understanding of the behavioral and neurochemical changes that are associated with MDMA voluntary intake. These assessments will allow us to examine DA and 5-HT responses after MDMA intake, using doses that are relevant to human MDMA use. Additionally, the analysis of brain tissue samples in naïve and experienced animals will provide insights on the possible neuroadaptational process that might occur in specific brain regions associated with drug addiction.

## Main DA and 5-HT Pathways in the Rat Brain




Thermoregulation:

The preoptic area and the anterior hypothalamus (Hyp) play an important role in the regulation of core temperature. In addition sympathetic premotor neurons in the rostral ventrolateral medulla and serotonin neurons in the raphe nuclei (RnR) control the vasomotor drive of a wide range of tissues to allow heat loss or heat conservation (for more detailed information refer to the thermoregulatory section in the general background).

Reinforcement:

The mesolimbic and mesocortical DA systems arise from the ventral tegmental area (VTA) and project to the nucleus accumbens (NAcc) and prefrontal cortex (PFC), respectively. The mesocorticolimbic DA system is thought to play an important role in

the initiation and maintenance of goal directed and reward mediated behaviors, as well as drug reinforcement. This system projects to other elements of the limbic system including the amygdala (Amyg) and hippocampus (Hip) and receives glutaminergic afferents from them. It is also in close proximity with the striatum (Str). In fact some neuroanatomist call this region the limbic striatum.

Locomotor behavior:

Various brain networks have been implicated in the regulation of motor behaviors. In general, the nigrostriatal dopamine pathway is concerned with the initiation and maintanence of motor behaviors. This pathway runs from the substantia nigra (SN) to the striatum (Str), and there is a feedback inhibition loop that includes acetylcholine, and GABA, and brain structures such as the motor cortex (MC), the globus pallidus, the thalamus (Thal) and the subthalamic nuclei. In the nucleus accumbens, dopamine-induced hyperactivity is thought to be induced by its inhibitory effect on GABAergic neurons projecting to the ventral globus pallidus. In addition, disruption of the normal serotonergic tone in animals can affect their exploratory motor behavior. Note that the serotonin pathways arise from the rostral and caudal raphe nuclei (RnR and CnR) and project to almost the entire brain. These nuclei are situated along the entire length of the brain stem and facilitate information processing from ascending sensory inputs.

# AGE-DEPENDENT (+)-MDMA-MEDIATED NEUROTOXICITY IN MICE


## ABSTRACT

In the present study the effects of a neurotoxic regimen of (+)-MDMA (20 mg/kg X 4, s.c.) in 4- and 10-week old C57Bl/6J mice during treatment and 7 days post-treatment were examined. Rectal temperatures monitored between (+)-MDMA injections (30 min post-injection/2 hr intervals) revealed hyperthermic responses in both age groups, with the magnitude of the response significantly greater in older mice. Seven days post-treatment, immunoblot analyses of the vesicular monoamine transporter 2 (VMAT2), and tyrosine hydroxylase (TH) revealed significant reductions (-37% and -58%, respectively) in the older animals, but not in the younger group, compared to age-matched controls. Dopamine transporter (DAT) expression was significantly reduced in both 4- and 10-week old animals (26%, and 69.7%, respectively). (+)-MDMA-treated animals also exhibited significantly lower levels of striatal dopamine, and 3,4-dihydroxyphenylacetic acid than controls, again the effect being more pronounced in the older animals. Although both age groups showed evidence of (+)-MDMA-induced toxicity, our data revealed that older animals exhibited a greater hyperthermic response to (+)-MDMA and were also are more susceptible to subsequent dopaminergic damage than the younger animals.

## INTRODUCTION

3,4-Methylenedioxymethamphetamine (MDMA) is an amphetamine analog and the major component of the street drug "Ecstasy". MDMA administered to experimental animals causes acute release of neurotransmitters, such as dopamine (DA) and serotonin (5-HT) in several brain regions, including the cortex, hippocampus, and striatum (Schmidt, 1987; Gough et al., 1991; Gudelsky and Nash, 1996). Long-lasting reductions of neural biomarkers indicate MDMA is a potent neurotoxicant that primarily targets serotonergic nerve terminal axons in rats and non-human primates. However, the mechanism of MDMA-induced terminal damage is likely to be different in rats and mice. In particular, MDMA toxicity appears to be limited to DA producing neurons in mice (Stone et al., 1987; Logan et al., 1988; O'Callaghan and Miller, 1994), although the basis for this species difference remains unknown. Also, mice appear to be less susceptible to MDMA-induced neurotoxicity than rats since higher doses of MDMA are required to produce long-term neuronal changes (Logan et al., 1988). In mice, MDMA produces significant reductions in DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in the biosynthesis of DA (O'Callaghan and Miller, 1994). In addition, there is strong evidence that MDMA causes nerve terminal degeneration in mouse striatum (Logan et al., 1988; O'Callaghan and Miller, 1994; Miller and O'Callaghan, 1995; Jayanthi et al., 1999; O'Shea et al., 2001).

MDMA induces hyperthermia when administered peripherally (Nash et al., 1988; Malberg and Seiden, 1998; Mills et al., 2003). Variations in ambient temperature that alter body temperature can modulate the extent of MDMA-mediated neurotoxicity. For example, when animals are restrained, or maintained at cooler temperatures, a partial

protection against MDMA toxicity is observed (Miller and O'Callaghan, 1995). Likewise, pharmacological treatments that reduce core temperature attenuate the toxicity of MDMA (Farfel and Seiden, 1995; Malberg et al., 1996). However, since pharmacological protection against MDMA-induced serotonergic neurotoxicity can be independent from changes in body temperature (Schmidt et al., 1990; Malberg et al., 1996), the precise relationship between MDMA-mediated neurotoxicity and increased body temperature remains unclear.

In contrast to the proportion of data reporting serotonergic neurotoxicity of MDMA, relatively few studies have focused on interactions between MDMA and the dopaminergic system. Indeed, no previous study has examined the effects of MDMA exposure on striatal dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2) expression in mice. Both DAT and VMAT2 are targets of drugs that damage the dopaminergic system, such as methamphetamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Therefore, reductions in DAT and VMAT2 levels correlate with axonal loss in several models of dopaminergic injury (Gainetdinov et al., 1998; Miller et al., 1999; Guilarte et al., 2003). The plasmalemmal DAT is an integral component of the dopaminergic neurotransmitter system, facilitating the uptake of extracellular DA into presynaptic DA nerve terminals. The DAT inhibitor, GBR 12909, prevents long-term MDMA-mediated dopaminergic toxicity in pretreated mice (O'Shea et al., 2001), suggesting that DA uptake may facilitate axonal loss after MDMA-induced DA release. Since VMAT2 promotes the storage of cytoplasmic DA into presynaptic vesicles (Erickson and Varoqui, 2000), it may be an important component of neurotoxicity prevention by sequestering excess cytoplasmic DA and other putative neurotoxicants within the neuron.

Younger animals appear to be less susceptible to the chronic biochemical changes induced by methamphetamine (Miller et al., 2000; Kokoshka et al., 2000; Iman and Ali, 2001), or MDMA (Broening et al., 1995). While it has been shown that age-dependent serotonergic toxicity of MDMA correlates with the magnitude of the hyperthermic response in rats (Broening et al., 1995), no previous studies have examined age-related changes in MDMA-induced DA neurotoxicity in mice. In the present study, we examined mice of two different ages (4- and 10-week old) and compared the effects of (+)-MDMA on body temperature and on changes in dopaminergic markers, including brain tissue levels of DA, 5-HT and their metabolites and striatal DAT, VMAT2 and TH expression. Acute effects of (+)-MDMA on striatal DA release in 10-week old mice were also examined.

## MATERIALS AND METHODS

### Animals and drug administration

Male C57Bl/6J mice (The Jackson Laboratory; Bar Harbor, ME) were used in this study. Two different age groups (4- and 10-wk old mice) were chosen to represent two distinct developmental groups (early adolescence and young adulthood) that are both subject to MDMA abuse in the human population. This strain of mice was selected since the most comprehensive characterization of MDMA-mediated dopaminergic toxicity has been done in C57Bl/6 mice (O'Callaghan and Miller, 1994; Colado et al., 2001; Gesi et al., 2004; Johnson et al., 2004). Animals were individually caged in temperature-controlled rooms (24-25°C), and provided with tap water and food *ad libitum.* (+)-MDMA HCl (NIDA, Bethesda, MD) was dissolved in 0.9% sodium chloride (saline), and administered in a total volume of 10 ml/kg. For the neurotoxic regimen, mice were

administered (+)-MDMA (20 mg/kg, s.c.), or saline, at 2 hr intervals for a total of four injections. A separate group of 10-wk old mice were injected with one (+)-MDMA injection (20 mg/kg, s.c.) to determine the acute DA response using *in vivo* microdialysis techniques. The (+) isomer of MDMA was selected for this study, since it appears to be the primary contributor to the neurotoxic effects of the drug (Johnson et al., 1988). All procedures were conducted in accordance with the Guide For The Care And Use Of Laboratory Animals (U.S. Public Health Service, National Institute of Health), and this research protocol was reviewed and approved by the University of Texas IACUC Committee.

**Rectal temperature measurements**

Rectal temperatures of saline and (+)-MDMA-treated animals were measured using a V911 digital thermometer (Procter & Gamble, Cincinnati, OH). Temperatures were recorded automatically in less than 7 seconds. Measurements were taken 30 minutes following each injection.

**Determination of mouse brain tissue monoamine levels**

Striatum and hippocampus dissected from saline and (+)-MDMA treated mice were sonicated in 0.1 M perchloric acid containing 347 μM sodium bisulphite, and 134 μM ethylenediaminetetraacetic acid (EDTA) disodium salt. Homogenates were centrifuged at 16,000 xg for 20 min and the supernatant removed, centrifuged, and analyzed for levels of DA, DOPAC, HVA, 5-HT and 5-HIAA by high performance liquid chromatography with an electrochemical coulometric array detector (HPLC-EC). Monoamines were measured at +150 mV and +300 mV. The mobile phase consisted of 4

mM citric acid monohydrate, 8 mM ammonium acetate, 54 μM EDTA disodium salt, 230 μM 1-octanesulfonic acid sodium salt monohydrate and 5% methanol (pH 2.5). The concentrations of neurotransmitters and metabolites were determined by comparison with calibration curves prepared from authentic monoamine standards (Sigma-Aldrich Corp., St. Louis, MO).

**Western Blotting**

Striatum dissected from saline- and (+)-MDMA-treated mice were sonicated in a homogenization buffer (320 mM sucrose, 5 mM N-(2-hydroxyethyl)piperazine-N-(2-ethanesulfonic acid) (HEPES), 1 μg/ml leupeptin, 1 μg/ml aprotinin, and 1 μg/ml pepstatin). Homogenates were centrifuged at 5,000 xg for 5 minutes (4˚ C), and the supernatant fraction subsequently centrifuged at 30,000 xg for 30 minutes (4˚ C). The resulting pellet was re-suspended in the homogenization buffer and protein concentrations were determined with a Bio-Rad protein assay, using bovine serum albumin as a standard. Protein samples were resolved in 10% polyacrylamide gels (Novex NuPAGE system; Invitrogen, Carlsbad, CA) after adding a 4X loading dye (200 mM Tris, 400 mM dithiothreitol, 8% glycerol, 0.4% bromophenol blue). Proteins were transferred electrophoretically to a polyvinylidene difluoride membrane, and non-specific binding sites were blocked with 5% nonfat dry milk in Tris-buffered saline (135 mM NaCl, 2.5 mM KCl, 50 mM Tris, and 0.1% Tween-20, pH 7.4). Transferred proteins were detected with a rat anti-DAT monoclonal, a rabbit anti-VMAT2 polyclonal, or a rabbit anti-TH polyclonal antibody (all from Chemicon International, Inc., Temecula, CA) in Tris-buffered saline with 2% nonfat milk. For detection, a goat antibody to rat IgG, or a goat antibody to rabbit IgG (ICN-Cappel Pharmaceuticals, Inc., Costa Mesa, CA) were utilized and coupled to enhanced chemiluminescence (SuperSignal® West Dura

Extended Duration Substrate, Pierce Chemical, Rockford, IL). Densitometric analyses were performed, and calibrated to co-blotted dilutional standards from control animals. Blots were then stripped for 20 minutes at 80˚C (8 M urea, 100 mM 2-mercaptoethanol, and 62.5 mM Tris, pH 6.8) and re-probed with an antibody to actin (Oncogene Research Products, San Diego, CA) to ensure equal loading.

**Stereotaxic surgery**

Mice were anesthetized prior to surgery with a chloral hydrate (42.5 mg/ml) and sodium pentobarbital (10.5 mg/ml) mixture at a dose of 3 ml/kg. In-dwelling guide cannulae (Plastics One, Roanoke, VA) were implanted above the striatum using a flat skull position and the following coordinates in relation to Bregma: AP: -0.62 mm; ML: 1.75 mm; DV: -2 mm. A dummy cannula (Plastic Ones, Roanoke, VA) was inserted in the guide cannula to prevent debris accumulation within the lumen. Animals were allowed to recover from surgery for 5-7 days before further experimental procedures were performed.

**Dialysis probes and *in vitro* probe recovery analysis**

Microdialysis probes were constructed using PE 20 tubing as the inlet and fused silica tubing (75 $\mu$m i.d.) inserted into a 2.5mm section of cellulose (13 kDalton cut-off; Spectrum, Houston) fitted through a 26 gauge internal cannula (Plastics One). Epoxy glue was used to seal both ends of the dialysis membrane. The outlet consisted of fused silica coated with PE 50 tubing. To determine recovery rates for each probe, Hamilton syringes were filled with freshly prepared filtered artificial cerebral spinal fluid (ACSF) solution, and pumped continuously through the probe at a rate of 1.63 $\mu$l/min. Probes used for

determining striatal levels of DA were place in a beaker containing ascorbate (1%), and 4 nM DA maintained at 37°C. 10-min dialysis samples from each probe were then collected, and assayed by HPLC coupled to ampirometric detection (Shizeido Capcell, 1.5 mm X 50 mm, 3 mm particle size column, ESA 5041 ampirometric cell detector with the oxidizing potential set to 200 mV; ESA, Inc., Chelmsford, MA). The mobile phase consisted of 150 mM Na2H2PO4, 50 mM EDTA disodium salt, 4.76 mM citric acid, 4.5–6.0 mM sodium dodecyl sulfate, 12.5% (v/v) acetonitrile, 12.5% (v/v) methanol, pH 5.6, constant flow rate of 0.2 ml/min. Probe recovery was calculated by comparing the peak heights of each dialysate and those from a 25% recovery standard solution.

Probes used for determining striatal MDMA levels were placed in a beaker containing ascorbate (1%), and 5$\mu$M (+)-MDMA dissolved in the ACSF solution maintained at 37°C. Two 30-min microdialysis samples were collected from each probe, and assayed by HPLC with coulometric detection (ESA Inc., Chemsford, MA; using an HR-80 column of 4.6 mm x 8 cm, and working electrode potential setting = 600 mV). The mobile phase consisted of 25 mM potassium phosphate monobasic and 10% acetonitrile (final pH 2.5, constant flow rate of 1ml/min). Probe recovery was calculated by comparing the peak heights of each dialysate with a 25% recovery standard solution.

**Extracellular DA detection**

Mice were briefly anesthetized with 1.5% isoflurane while the microdialysis probe was lowered through the indwelling cannula. Artificial CSF was then pumped through the probe at a rate of 0.2 $\mu$l/min for approximately 12 hours. The pump speed was then increased to 1.65 $\mu$l/min, and one hour later, the first 30 min basal sample was collected. For determination of MDMA-induced DA levels, animals were injected once

with either saline or (+)-MDMA (20 mg/kg, s.c.). Three 30 minute basal samples were collected before treatment and eight more samples after the injection. Dialysate DA content was assessed using HPLC coupled to amperometric detection (as described above). The amount of DA within each sample was determined by comparison with standards prepared and analyzed on the day of sample analysis. Data were collected and analyzed using an ESA Model 500 Data station.

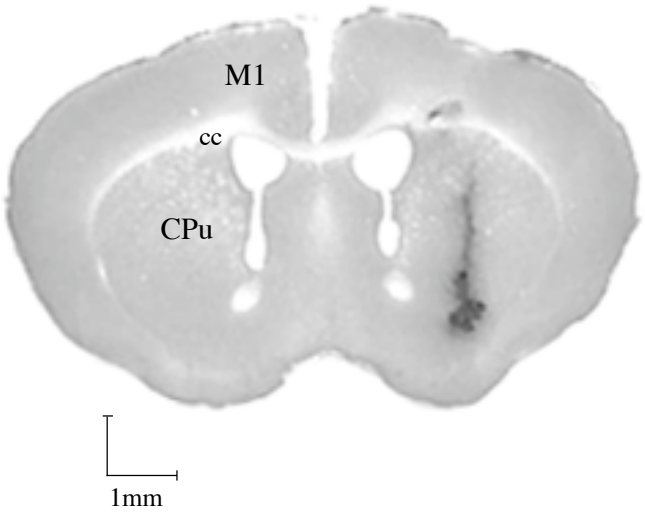
**Extracellular MDMA detection**

Mice were briefly anesthetized with 1.5% isoflurane while microdialysis probe was lowered through the indwelling cannula. Artificial CSF was then pumped through the probe at a rate of 0.2$\mu$l/min for approximately 12 hours. The pump speed was then increased to 1.65$\mu$l/min, and one hour later, a 30 min basal sample was collected. For determination of MDMA striatal levels, animals were then injected 20 mg/kg, s.c. (+)-MDMA. Control animals received equal volumes of vehicle. A series of 30-min dialysate samples were collected from freely moving animals for two hours. Striatal levels of (+)-MDMA were determined using HPLC analyses. Quantification was made by reference to calibration curves made with (+)-MDMA standard solutions.

**Histology**

After completion of the experiment, animals were euthanized, and microdialysis probes placement into the striatum was confirmed with histological analyses of 60 µm coronal sections stained with cresyl violet (see Fig. 1.1).

Figure 1.1 Representative cresyl violet stained section of the mouse brain. The figure shows the correct placement of the microdialysis probe in the left striatum. M1: primary motor cortex; cc: corpus callosum; Cpu: caudate putamen (striatum)



### Data analysis

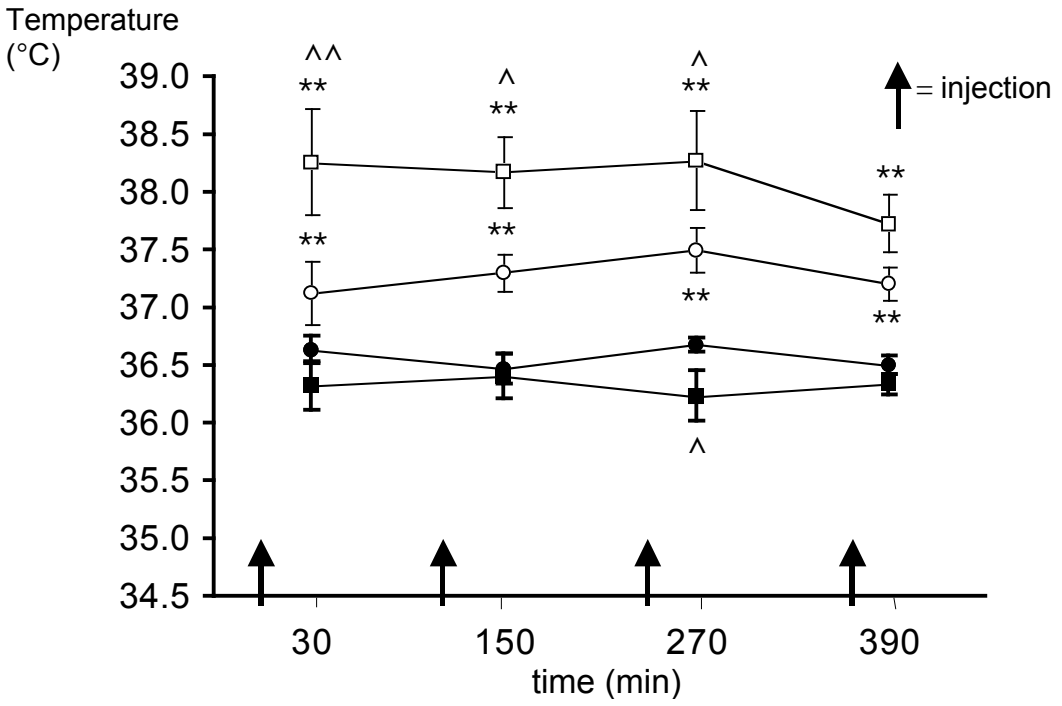
The data are presented as mean ± SEM values. Total tissue content (ng/mg) of DA, and 5-HT, and their metabolites were analyzed using two-way ANOVA tests (randomized blocks). Protein expression values for the Western blot data were obtained as integrated densitometric values and transformed by linear regression to mg protein using dilutional standards. Data was analyzed as mg protein levels using two sample t-tests for each age group (MDMA vs. saline). Dialysate DA levels were converted from pg/ml concentrations to percent of baseline for ANOVA analyses. Two-way ANOVAs with repeated measures were used to analyze the temperature and DA dialysate data. Posthoc tests (Fishers LSD) were performed to determine specific group differences when overall ANOVA results showed significant group, time or treatment differences.

## RESULTS

### MDMA treatment/ Rectal temperature

To minimize stress associated with rectal temperature assessment, the effect of MDMA treatment on rectal temperatures was determined by comparing MDMA-receiving animals with age-matched saline-treated groups. Compared to saline-treated animals, (+)-MDMA treatment resulted in significantly higher rectal temperatures in both the 4- and 10-wk old mice. Temperatures of the (+)-MDMA-treated animals were elevated 30 minutes after the first injection, and remained significantly elevated for the duration of the experiment. In addition, the hyperthermic response was significantly higher in the older animals compared to the younger animals at all but the last measurement recorded (see Fig. 2.1).

Figure 2.1 Acute (+)-MDMA effects on body temperature in 4- and 10-wk old mice. Rectal temperature was recorded 30 minutes after each saline or (+)-MDMA (20 mg/kg, s.c.) injection. Animals were administered four injections at two-hr intervals (see ↑). Results are shown as mean ± SEM for 4-wk saline- (●, n=14), 4-wk MDMA- (O, n=14), 10-wk saline- (■, n=6), and 10-wk old MDMA-treated animals (□, n=5). ** = significant difference between (+)-MDMA- and saline-treated animals in same age groups at $p < 0.01$. ^^, ^ = significant difference between 4- and 10-wk old animals receiving same treatments at $p<0.01$ and $p<0.05$, respectively.



**MDMA treatment/ Monoamines and metabolite levels in striatum and hippocampus**

Seven days after (+)-MDMA treatment, 4-wk old animals showed DA, DOPAC and HVA levels that were significantly lower than saline-treated animals of the same age (DA, $p<0.01$; DOPAC and HVA both $p<0.05$). Striatal 5-HT and 5-HIAA content remained comparable between the 4-wk old (+)-MDMA- and saline-treated animals. In the older mice, MDMA administration resulted in significantly lower DA, DOPAC and 5-HT levels ($p<0.01$ for all) as compared to same-age controls. Though DA striatal tissue content was comparable in saline-treated 4- and 10-wk old animals, the older group showed significantly lower levels of striatal DA 7-days post-MDMA administration compared to the MDMA-treated 4-wk old animals. No MDMA-induced changes in the levels of hippocampal 5-HT or 5-HIAA were observed in either age group (see Table 1.1).

Table 1.1 Chronic (+)-MDMA effects on striatal and hippocampal monoamines and metabolites content in 4- and 10-wk old mice. Striatal and hippocampal content of DA, DOPAC, HVA, 5-HT, and 5-HIAA expressed as ng/mg wet tissue 7 days after saline, or (+)-MDMA (20 mg/kg) s.c. injection regimen (total of 4 injections at two hour intervals). Data are shown as mean ± SEM for 4-wk Saline- (n=7), 4-wk MDMA- (n=7), 10-wk Saline- (n=6), and 10-wk old MDMA-treated animals (n=5). **p<0.01, *p<0.05 for MDMA vs. Saline in the same age group. ^^ = significant difference at p<0.01 between 4-wk MDMA- vs. 10-wk MDMA-treated animals. Italicized values indicate significant differences between different-aged control groups.

| | 4 WEEK OLD | | | 10 WEEK OLD | | |
|---|---|---|---|---|---|---|
| **Striatal** | **Saline** | **MDMA** | **% diff** | **Saline** | **MDMA** | **% diff** |
| DA | 9.08 ± 0.38 | 6.87 ± 0.59** ^^ | -24.0% | 7.69 ± 0.63 | 1.00 ± 0.21** | -86.99% |
| DOPAC | 1.45 ± 0.18 | 0.97 ± 0.04* | -33.10% | 1.93 ± 0.23 | 0.57 ± 0.17** | -70.46% |
| HVA | 1.10 ± 0.14^^ | 0.84 ± 0.06*^^ | -23.63% | 0.45 ± 0.05 | 0.22 ± 0.04 | -51.11% |
| 5-HT | 0.33 ± 0.03^^ | 0.36 ± 0.02^^ | 9.09% | 0.56 ± 0.03 | 0.45 ± 0.01** | -19.64% |
| 5-HIAA | 0.27± 0.01^ | 0.27 ± 0.01^^ | 0.00% | 0.43 ± 0.02 | 0.46 ± 0.11 | 6.97% |
| **Hippocampal** | | | | | | |
| 5-HT | 0.38±0.03^^ | 0.36 ± 0.03^^ | -5.26% | 0.23 ± 0.05 | 0.19 ± 0.01 | -17.39% |
| 5-HIAA | 0.39± 0.02 | 0.36 ± 0.03 | -7.69% | 0.43 ± 0.06 | 0.52 ± 0.07 | 20.93% |

**MDMA treatment/ DAT, VMAT2, and TH expression levels**

Compared to saline-treated animals, 4-wk old mice showed significantly lower DAT-immunoreactivity (-26%) seven days after (+)- MDMA exposure ($p < 0.01$; see Fig 3.1A and B). However, reductions in DAT expression levels were more pronounced in the older animals (-69.7%, $p < 0.01$; see Fig. 3.1A and B). Western blot analysis of VMAT2 protein in striatum (Fig. 3.1A & 4.1A) showed the characteristic three bands at 45, 55, and 75 kDa, being the truncated, native and glycosylated forms of the transporter, respectively (Miller et al., 1999). While, VMAT2 and TH expression was comparable between younger animals treated with either saline or (+)- MDMA seven days post-treatment, both VMAT2 and TH protein levels were significantly lower (-37.3% and -57.7%, respectively, $p<0.01$ for both) in older mice treated with (+)- MDMA compared to same-age control animals (see Fig 3.1 and 4.1).

Figure 3.1 and 4.1 Chronic (+)-MDMA effects on DAT, VMAT2, and TH expression. 3.1A and 4.1A) Representative blots of DAT, VMAT2, and TH immunoreactivity in 4-wk (2A) and 10-wk (3A) animals in samples from pooled control, and (+)-MDMA-treated groups. Lanes 1-5, Standard curve from saline-treated mouse striatum (2.5, 5, 10, 15, and 20 mg, respectively). Lane 6, saline-treated (10 mg). Lane 7, 20 mg/kg X 4 (+)-MDMA-treated (10 mg). 3.1B and 4.1B) Data are presented as DAT, VMAT2, and TH expression levels (mg protein) in saline- and (+)-MDMA-treated groups. 3B) 4-wk: Graphs represent the mean ± SEM, for DAT/Saline (□, n=7), DAT/MDMA (■, n=7), VMAT2/Saline (□, n=6), VMAT2/MDMA (■, n=7), TH/Saline (□, n=6), and TH/MDMA (■, n=7); ** = significant differences at $p < 0.01$. 4B) 10-wk: Graphs represent the mean + SEM, for DAT/Saline (□, n=6), DAT/MDMA (■, n=5), VMAT2/Saline (□, n=6), VMAT2/MDMA (■, n=5), TH/Saline (□, n=6), and TH/MDMA (■, n=5); ** = significant differences at $p < 0.01$.

Figure 3.1

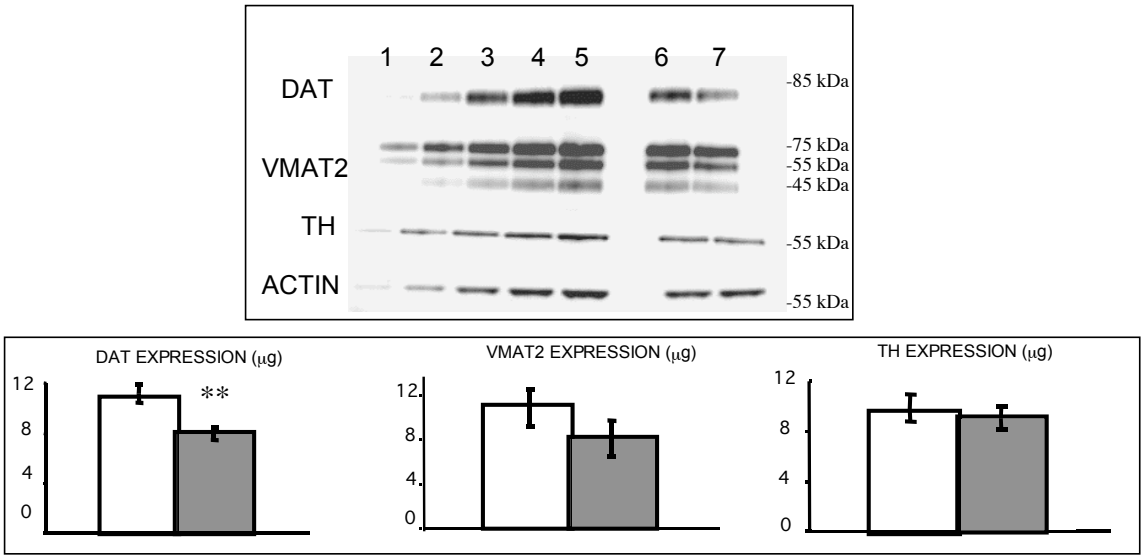
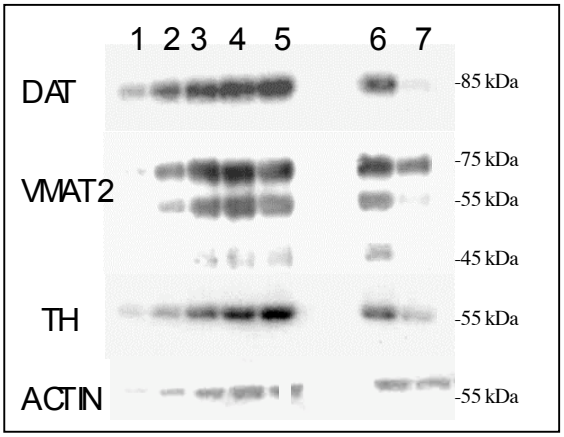


Figure 4.1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VMAT2
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-55 kDa
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TH
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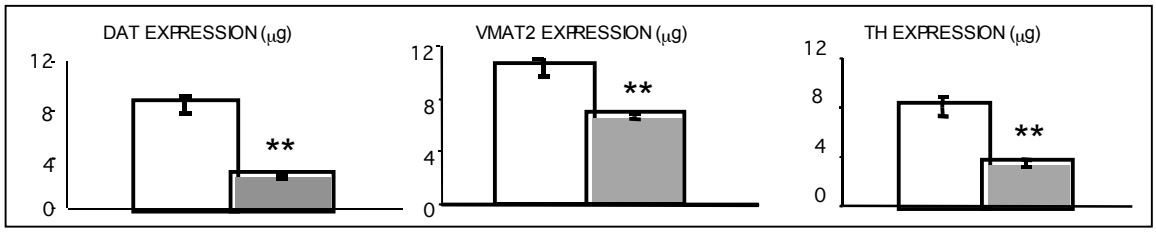
ACTIN
-55 kDa


DAT EXPRESSION (μg)
12
8
4
0
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VMAT2 EXPRESSION (μg)
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TH EXPRESSION (μg)
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4
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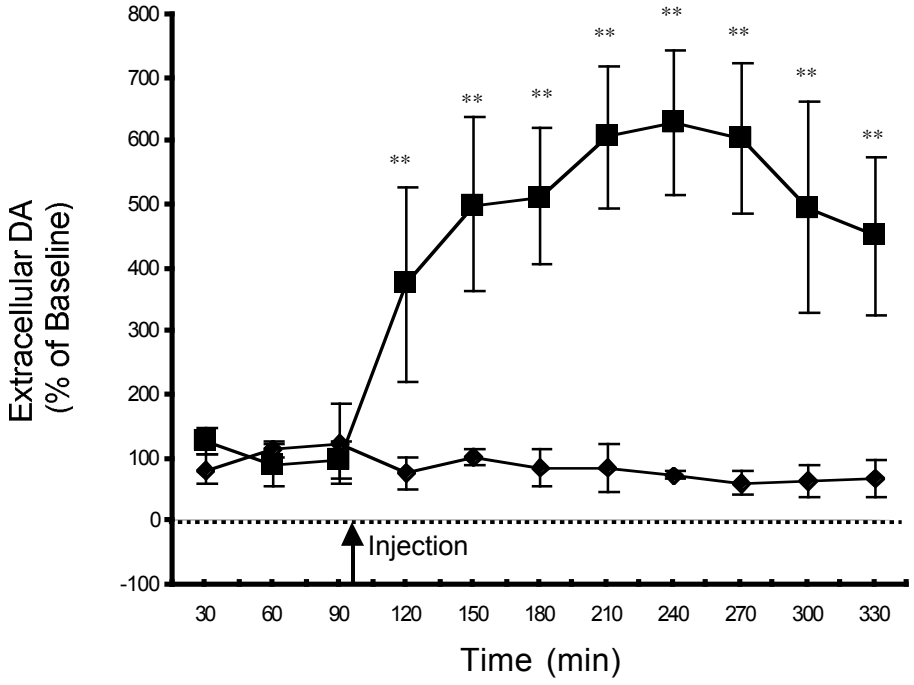
**Acute MDMA treatment/ Dialysate levels of extracellular striatal DA**

In 10-wk old mice, a single injection (20 mg/kg, s.c.) of (+)- MDMA dramatically increased striatal DA levels. DA levels peaked between 2-2.5 hrs post-injection and remained significantly higher than baseline and saline levels ($p<0.01$) for the entire 4 hr assessment period (see Fig. 5.1; basal levels of DA: 24.48 $\pm$ 3.0 pg/20 μl and 28.23 $\pm$ 4.4 pg/20 μl, for control and MDMA-treated animals respectively).

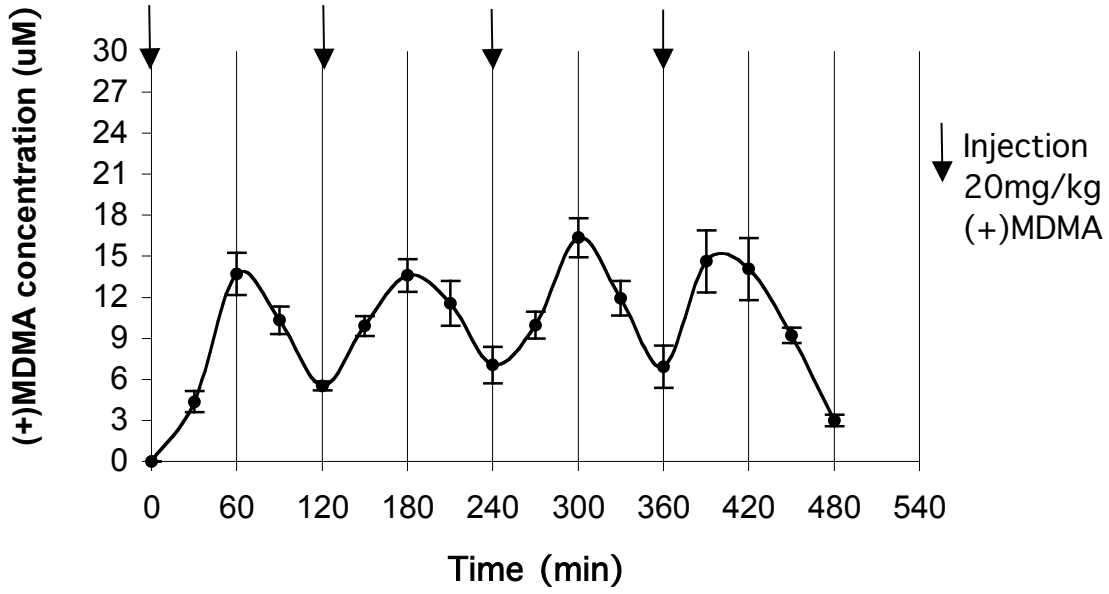
Figure 5.1 Acute effects of (+)-MDMA on striatal DA release/10-wk. Extracellular striatal DA levels before and after a single subcutaneous saline and (+)-MDMA (20 mg/kg, s.c.) injection. Data are expressed as percentage of pre-injection (baseline) samples (mean ± SEM) for saline- (♦, n=4-5) and MDMA-treated (■, n=4-5) animals. ** = significant difference between MDMA- and saline-treated animals at $p < 0.01$.



**Striatal extracellular levels of MDMA after systemic administration**

Levels of (+)-MDMA were detected in the extracellular space as soon as 30 minutes after the first injection of the drug, and peaked at about 1 hour (see Fig. 6.1). Subsequent injections increased (+)-MDMA levels although not significantly. An estimated maximum concentration of 13.4 μM was calculated after correcting for probe recovery (average probe recovery: 11.6%) by averaging the four peak concentrations that occurred at 1, 3, 5 and 6.5 hours.

Figure 6.1 Extracellular striatal levels of (+)-MDMA following subcutaneous administration of the drug in C57Bl/6J mice. Striatal extracellular (+)-MDMA was measured from microdialysis samples collected from (+)-MDMA-treated animals following a regimen of four injections of (+)MDMA (20mg/kg s.c. one injection every two hours).



## DISCUSSION

Though precise role of hyperthermia in MDMA-mediated toxicity is not fully understood, preventing hyperthermia in rats attenuates, or abolishes, the deleterious effects of MDMA (Miller and O'Callaghan, 1995; Farfel and Seiden, 1995; Malberg et al., 1996, O'Shea et al., 2002). However, neuroprotection can be also achieved without alterations in body temperature. For example, pretreatment with fluoxetine prevents MDMA-induced neurotoxicity in rats that is independent of changes in body temperature (Malberg et al., 1996). Likewise, pretreatment of mice with fluoxetine prevented the MDMA-associated hyperthermic response, but had no effect on MDMA-mediated DA damage (O'Shea et al., 2001). Consistent with previous work (O'Callaghan and Miller, 1994; Colado et al., 2001; Carvalho et al., 2002), the present study revealed MDMA-induced hyperthermia (see Fig. 2.1). The magnitude of this effect was significantly greater in older mice compared to the younger group, and correlated with significantly greater depletions of striatal DA and DOPAC (see Table 1.1) and decreased DAT, VMAT2 and TH a week after (+)-MDMA treatment (see Fig. 3.1). In rats, age-related differences in MDMA-mediated toxicity have been suggested to result from different hyperthermic responses developed at early and late postnatal ages (Broening et al., 1995). For example, young rats maintained at higher ambient temperatures develop thermic responses and chronic biochemical changes comparable to those observed in older animals when treated with methamphetamine (Cappon et al., 1997). In addition, age-linked increases in hyperthermia and dopaminergic toxicity have been reported in rats treated with methamphetamine (Imam and Ali, 2001). The present study suggests similar interactions between age, MDMA-induced hyperthemia and dopaminergic toxicity in mice.

Reduction of striatal DA generally corresponds with terminal degeneration, though drugs of abuse, and pharmacological treatments (e.g. methamphetamine, L-dopa) can also modulate DA levels (Reveron et al., 2002; Moszczynska et al., 2004). While the exact mechanism accounting for the reduction in DA nerve terminals remains unknown, repetitive large MDMA doses (e.g. 20, 30, or 40 mg/kg) elicit significant reductions in DA, DOPAC and HVA, and nerve terminal degeneration in the mouse striatum (Logan et al., 1988; O'Callaghan and Miller, 1994; Jayanthi et al., 1999; O'Shea et al., 2001). In addition, MDMA causes increases in glial fibrillary acidic protein (GFAP) immunoreactivity (Miller and O'Callaghan, 1995), an intermediate filament protein known to be a sensitive and early marker of neuronal damage (O'Callaghan, 1988). The present study reports that, compared to saline-treated mice of both ages tested, significantly lower levels of DA and DOPAC are observed seven days after (+)-MDMA-treatment. Decreased DA levels were significantly more pronounced in the older mice (see Table 1.1). In addition, for 4-wk but not 10-wk old mice, (+)-MDMA-treatment resulted in lower HVA levels, and lower 5-HT levels in 10-wk, but not 4-wk old animals. Precise reasons for seemingly incongruent (+)-MDMA-induced toxicity between the age groups in the present study are to be determined, yet it is conceivable that age-linked differences in drug effects might also be due to dissimilar levels of total, or extracellular DA at different developmental stages (Bolla et al., 1998). Though our results indicate no significant differences in the total content of striatal DA or DOPAC between 4- and 10-wk old mice (see Table 1.1), significant differences in striatal 5-HT, HVA and 5-HIAA levels between the 4- and 10-wk old control groups were observed in this report. Higher basal neurotransmitter levels between age groups may have favored statistical detection of HVA depletion in the younger group and 5-HT in the older group following (+)-MDMA treatment. Nevertheless, though we found decreased striatal 5-HT levels 7 days

after MDMA in older mice in the present study, 5-HT and 5-HIAA levels were unchanged in the hippocampus (see Table 1.1). Other work has shown similar reductions to be short-lived and recover 21 days post-treatment (O'Callaghan and Miller, 1994). Thus, the present findings are not incongruent with literature reporting the most prominent neurotoxic effects of MDMA target dopaminergic systems in mice (Green et al., 2003).

VMAT2-immunoreactivity is a robust and reliable marker of DA nerve terminal viability. For example, the magnitude of DA neurodegeneration has been shown to correlate with levels of VMAT2 expression (Tillerson et al., 2002), and VMAT2 reduction is associated with DA terminal damage (Hogan et al., 2000; Kilbourn et al., 2000). Both DAT and VMAT2 proteins play key roles in MDMA-induced dopaminergic toxicity, and the activity of both transporters is reduced following MDMA administration (Hansen et al., 2002, Bogen et al., 2003). However, reductions in DAT expression may also be indicative of temporal compensatory changes (Wilson et al., 1996; Kish et al., 2001; Hansen et al., 2002), whereas, previous studies indicate VMAT2 protein levels are not subject to neuronal modulation (Vander Borght et al., 1995; Wilson et al., 1996).

TH levels are also a commonly used marker of the dopaminergic nerve terminals. Decreased TH levels and activity have been reported after treatment with dopaminergic neurotoxicants (O,Callaghan and Miller, 1994; Ara et al., 1998). The present study showed concomitant decreases in DA concentrations (see Table 1.1) and reductions in DAT protein levels in 4- (Fig. 3.1) and 10-wk old (Fig. 4.1) mice after (+)-MDMA administration. Decreased levels of both DA and DAT were more pronounced in older mice. In addition, VMAT2 and TH expression levels decreased in older, but not

younger mice. Our current data therefore suggest that older mice are most vulnerable to neurotoxic effects of (+)-MDMA.

Previous work has shown that a single injection of (+/-)-MDMA (30 mg/kg, i.p.) causes a modest increase in mouse striatal DA, which is only enhanced after subsequent injections (Colado et al., 2004). Our data showing that striatal DA levels are significantly increased after only one (+)-MDMA (20 mg/kg, s.c.) injection is consistent with findings that (+)-isomer is a more potent DA releaser (Johnson et al., 1986; Hiramatsu and Cho, 1990).

Whether MDMA itself, dopamine release into the synaptic cleft, and/or a putative metabolite of MDMA are responsible for the long-term effects observed after MDMA administration remains unknown. MDMA metabolism has been poorly characterized in mice, and it is not clear whether different rates of metabolism or different metabolic pathways would explain the age-dependent neurotoxic profiles observed in this study. One possibility to explain the lack of dopaminergic damage in the younger group might be different MDMA pharmacokinetics in both groups, and although there have been no studies addressing this topic, higher levels of MDMA in the striatum do not necessarily translate to higher vulnerability to MDMA in mice (Johnson et al., 2004). In fact, MDMA alone is not responsible for the dopaminergic damage observed after peripheral MDMA administration since direct intrastriatal perfusion of MDMA did not cause striatal DA depletion (Escobedo et al., 2004). Dissimilar hyperthermic responses have been linked to developmental differences in toxicity reported in both rats and mice after the administration of various compounds such as methamphetamine, or MDMA (Imam and Ali, 2001; Cappon et al., 1997). However, hyperthermia alone does

not cause long-term dopamine reductions (Schmidt et al., 1990; Malberg et al., 1996). For this reason, several factors, such as divergent VMAT2/DAT activities, differences in extracellular DA levels and disparities in the magnitude of hyperthermic responses to MDMA might account for the lower sensitivity of younger animals to MDMA. In addition, though total protein levels of VMAT2 or DAT were shown to be similar between age groups in the present study, it is possible that membrane-bound levels of DAT, or the activity of these transporters might vary across ages. Even though no studies have investigated possible developmental changes in the dopaminergic system in mice, some work has been done in rats. In fact, transient expressions of VMAT2, SERT and TH have been observed in non-monoaminergic neurons in the rat during postnatal brain development (Berger et al., 1985; Lebrand et al., 1996; Hansson et al., 1998). These findings lead to the hypothesis that non-monoaminergic neurons might take up and store DA and/or 5-HT to reduce toxic levels during early development. These differences are however transients and no differences in the expression of these transporters are found after the second or third week of birth. Differences in the dopaminergic response to (+)-MDMA between age groups may also contribute to variability in hyperthermic and other responses. However, while extracellular DA levels from 10-wk old animals are reported here, this particular comparison was not made because of multiple logistic difficulties with performing *in vivo* microdialysis in the younger group (e.g. smaller and more fragile skulls). Our results also demonstrate (+)-MDMA reaches the striatum. Previous studies in rats have estimated a maximum hippocampal concentration of 20 μM after a single neurotoxic injection of (+/-)-MDMA (Esteban, et al., 2001). Using the (+)-MDMA isomer, our results indicate larger and repetitive doses of (+)-MDMA are needed to achieve a similar maximum concentration (16.4 μM) in the striatum (see Fig. 6.1).

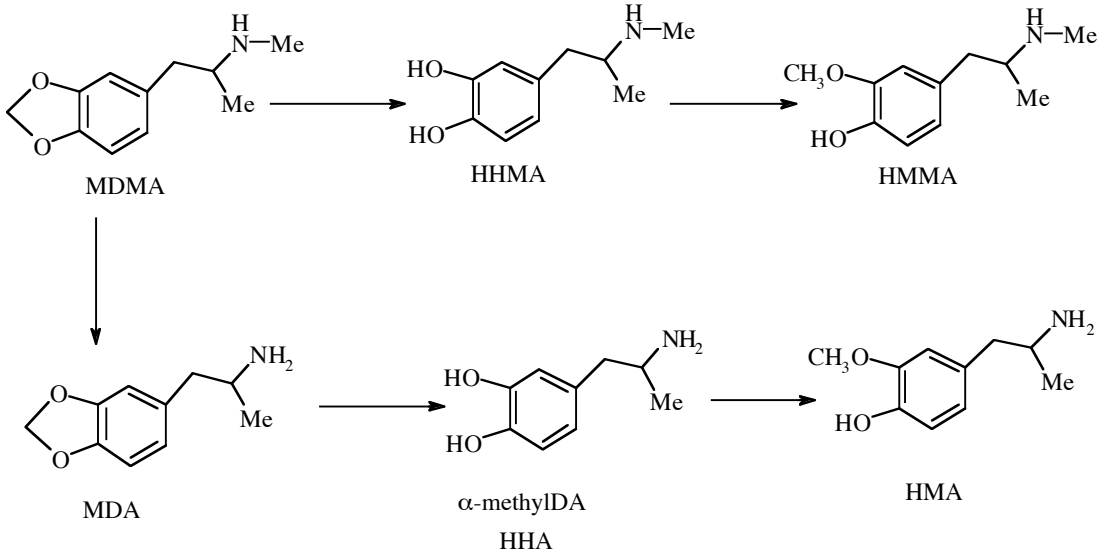
In conclusion, this study is the first to reveal age-dependent sensitivity to (+)-MDMA-mediated dopaminergic neurotoxicity in mice. Older mice exhibit an enhanced hyperthermic response to acute (+)-MDMA injections and are also more vulnerable to (+)-MDMA-induced depletions in striatal DA DAT, VMAT2 and TH levels one week after (+)-MDMA exposure. Though the associated increases in body temperature might be a factor in predisposing older mice to striatal damage, the contribution of MDMA-induced hyperthermia to MDMA-mediated DA toxicity in the mouse should be a topic for more thorough investigation.

## CHAPER 1 SUMMARY

This study has demonstrated the age-dependent sensitivity to MDMA-mediated dopaminergic toxicity in mice. While a neurotoxic regimen of MDMA caused significant reductions in striatal DA, and its metabolites, and decreased DAT immunoreactivity in younger animals, the same regimen induced more profound depletions in the same compounds, and decreased DAT, VMAT2 and TH striatal expressions in 10 week-old mice. Several possibilities might explain this age-dependent toxicity, and our data suggest the dissimilar effect observed in rectal temperatures might account for this difference. However, other factors should be taken into account.

The kinetics of MDMA have been studied in several animal species including rats and non-human primates, and in living humans (Maurer et al., 2000; Ricaurte et al., 2000). MDMA metabolism has been poorly characterized in mice, but several investigators have proposed systemic metabolism of MDMA is required to cause long lasting central damage (Esteban et al., 2001; Paris and Cunningham, 1992; Schmidt and Taylor, 1988). In humans, MDMA is metabolized in the liver to 3,4-dihydroxymethamphetamine (HHMA; see diagram below), primarily by two cytochrome p450 isoenzymes ($CYP_{2D6}$ (low Km component) and $CYP_{1A2}$ (high Km component)) and, to a minor extent, by the polymorphic isoforms $CYP_{2B6}$, and $CYP_{3A4}$ (Kraemer and Maurer, 2002; Kreth et al., 2000). HHMA is a highly reactive compound, and thus its presence has only been demonstrated *in vitro* (Hiramatsu et al., 1990; Kreth et al., 2000). The O-methylation of HHMA by cytosolic catechol-O-methyl-transferase (COMT) forms the major metabolite of MDMA in plasma and urine (4-hydroxy-3-methoxy-methamphetamine; HMMA). MDMA N-demethylation is catalyzed by $CYP_{2B6}$, although $CYP_{1A2}$ and $CYP_{3A4}$ might also participate. The resulting product, 3,4-

methylenedioxyamphetamine (MDA) can also undergo O-demethylenation to form 3,4-dihydroxyamphetamine (HHA or a-methyldopamine) and further metabolism by COMT to form 4-hydroxy-3-methoxyamphetamine (HMA).




CYPs are located in the endoplasmic reticulum (microsomal fraction) or mitochondrial fraction of mammalian tissues. Drug metabolizing CYPs are expressed at high levels in hepatic microsomal fractions, with lower levels in lung, kidney, gut and brain. Only one study has examined hepatic MDMA kinetics in mice (Steele et al., 1991). The authors found that rat hepatic microsomes produced higher levels of HHMA compared to mouse liver microsomes. In rats, the methyldioxy group of MDMA might interfere with the metabolism of this amphetamine derivative. To the contrary, side-chain metabolism in mice might contribute to MDMA clearance. The cytochrome p450 involved in MDMA demethylenation in rats is $CYP_{2D1}$ (the gene encoding for debrisoquine 4-hydroxylase). The homologous gene in mice has been mapped to the chromosome 15, and therefore, is also a $CYP_2$ gene. However, the exact identification among the mouse genes of this family is unknown (Gonzalez et al., 1987). It is important

to note that sex-dependent differences in CYP activities, and isoform population have been reported in rats and mice, as well as in different strains of mice (Mugford and Kedderis, 1998). This might explain the different degree of sensitivity to MDMA observed in different strains of mice (BALB/c, C57Bl/6J and CBA; (Zheng and Laverty, 1993). Hence, the age-dependent sensitivity to MDMA DA nerve toxicity might also be explained by differences in CYPs expression at different developmental stages. In fact, different CYPs transcripts have been detected in mouse fetus at various stages during development, and it is thought CYPs functions are required for the development of various organs (Choudhary et al., 2003; Choudhary et al., 2005; Libby et al., 2003). Additionally, $CYP_{1A1}$ and $CYP_{1A2}$ mRNA levels decreased approximately 40% between age 4-5 months and 31 months in mice (Mote et al., 1991). Further studies aiming to investigate developmental changes in CYPs involved in MDMA metabolism would help to clarify the basis for MDMA age-dependent toxicity in mice.

Estrogen has been shown to regulate the expression of different DA and 5-HT receptors and transporters (Zhou et al., 2002). Hence, another possibility might be that differences in estrogen content at the onset and during puberty might result in dissimilar MDMA mechanisms of actions in 4 and 10 week-old mice. Supporting this hypothesis, amphetamine-induced DA release and accompanied hyperactivity vary with the estrous cycle as E2 and p levels change (Becker and Cha, 1989), and a recent study suggested estrogen might play a role in MDMA-induced hyperactivity and DA release (Zhou et al., 2003). Hence, quantification of plasma estrogen levels in 4- and 10-week animals would provide further information about MDMA age-dependent sensitivity.

We also have to consider that synaptogenesis in various regions of the brain increases from the neonatal period to puberty (Arai and Matsumoto, 1978; Caley and

Maxwell, 1968a; Caley and Maxwell, 1968b). Over the pre-pubertal period, imaging studies have shown changes in the density of DA and 5-HT transporters (Lidow et al., 1991; Zecevic et al., 1989). Furthermore, the length of axons containing TH progressively increases until adulthood in non-human primates (Rosenberg and Lewis, 1995). Several reports have demonstrated dopamine levels in the rat brain increase before and around the age of puberty (Goldman-Rakic and Brown, 1982; Hohn and Wuttke, 1979) while levels of 5-HT remain approximately the same. In fact, postnatal changes in the monoaminergic inputs to cortical structures underlie the basis of learning and memory development in mammals. Since DA itself might play an essential role in MDMA-induced neurotoxicity, increased brain levels of DA might explain the higher susceptibility observed in this study in 10 week-old mice.

# EXPERIENCED-DEPENDENT CHANGES IN TEMPERATURE AND BEHAVIOR INDUCED BY MDMA


## ABSTRACT

Hyperthermia and hyperlocomotor activity are commonly reported acute effects of high dose, experimenter-delivered 3,4-methylenedioxymethamphetamine (MDMA). The current investigation was performed to determine short- to long-term physiological and behavioral changes induced by moderate intake MDMA self-administration. In the present study, rats self-administered MDMA (approx. 2.0 – 7.0 mg/kg/day) across 20 days during daily 2-hr operant sessions. Locomotor activity was assessed during MDMA self-administration sessions and core temperatures were recorded before and after each session. Findings of the first several sessions showed core temperatures significantly decreased after MDMA self-administration compared to baseline and to a control group that self-administered saline during operant sessions. As sessions proceeded, the MDMA-induced hypothermic response diminished, and core temperatures rose significantly by the last few sessions. Also, locomotor activity during MDMA self-administration sessions was initially equivalent to saline level activity, but increased by Day 8 to significantly greater levels. Our findings demonstrate experience-dependent changes after voluntary administration of MDMA that are clearly observable in temperature regulation and behavioral activity.

## INTRODUCTION

The psychostimulant 3,4-methylenedioxymethamphetamine (MDMA) is the main component of the designer drug known as “ecstasy”. Substantial research has demonstrated that MDMA affects body temperature in humans and experimental animals (Carvalho et al., 2002; Fantegrossi et al., 2003; Freedman et al., 2005; Malberg and Seiden, 1998; Mills et al., 2003; Nash et al., 1988; O'Shea et al., 1998; Piper et al., 2005). Animal studies show MDMA-associated changes in body temperature are influenced by several factors including age, ambient temperature, water availability and housing conditions (Dafters, 1995; Dafters and Lynch, 1998; Gordon and Fogelson, 1994; Malberg and Seiden, 1998; Reveron et al., 2005), and many report that MDMA administration increases core temperature (Broening et al., 1995; Malberg et al., 1996; Nash et al., 1988; O'Shea et al., 1998). Clinical research demonstrates recreational doses of MDMA (1.5–2.0 mg/kg, p.o.) also cause hyperthermia (Freedman et al., 2005; Liechti et al., 2000), though increases in human body temperature do not appear to be affected by ambient temperature (Freedman et al., 2005). However, rather than producing unidirectional increases in body temperature, evidence indicates that MDMA interferes with general mechanisms of thermogenesis. For example, under certain circumstances, MDMA administration results in hypothermia (Malberg and Seiden, 1998; Marston et al., 1999; O'Shea et al., 2005; Piper et al., 2005) as well as hyperthermia (Broening et al., 1995; Malberg et al., 1996; Nash et al., 1988; O'Shea et al., 1998) and sensitization to MDMA-induced hyperthermic effects (Dafters, 1995; Spanos and Yamamoto, 1989).

MDMA produces behavioral effects characteristic of the serotonin syndrome (Green et al., 1995; Hiramatsu et al., 1989; Spanos and Yamamoto, 1989), including an acute dose-dependent increase in locomotor activity in rodents (Callaway et al., 1990;

Gold et al., 1988). In addition, MDMA can engender behavioral sensitization (Kalivas et al., 1998; Ramos et al., 2004; Spanos and Yamamoto, 1989), a property shared by other psychostimulant drugs, characterized by enhanced behavioral responses following repetitive exposure. MDMA administration causes the release of serotonin and dopamine, both *in vivo* and *in vitro* (Callaway et al., 1990; Crespi et al., 1997; Gudelsky and Nash, 1996; Wichems et al., 1995), and it has been suggested that both neurotransmitter systems may mediate MDMA-induced changes in body temperature (Mechan et al., 2002; Shankaran and Gudelsky, 1999) and locomotor activity (Bankson and Cunningham, 2001; Bankson and Cunningham, 2002; Gold et al., 1989).

Many studies have investigated effects of high dose experimenter-administered MDMA on locomotor activity and core temperature in animals (Carvalho et al., 2002; Dafters and Lynch, 1998; Kalivas et al., 1998; Marston et al., 1999; Mechan et al., 2002; Shankaran and Gudelsky, 1999) or "binge" amounts of MDMA to investigate MDMA-mediated neurotoxicity and hyperthermia (Green et al., 2004; Sanchez et al., 2004). However, to examine physiological and behavioral consequences of MDMA during voluntary intake at dosages relevant to recreational use, operant behavior models are a useful methodology. In the present study, short- and long-term effects of moderate MDMA intake on core temperature and behavioral activity were examined in rats self-administering MDMA on a daily basis.

## MATERIALS AND METHODS

Male Sprague-Dawley rats (4 weeks, Charles River Laboratories, Inc. Wilmington, MA) were housed in a room with reverse light cycle (lights off 7:00am-7:00pm). The ambient temperature of the animal colony was maintained at 22 ± 1°C.

Animals were kept in clear cages and water and laboratory food pellets were available *ad libitum*. To minimize stress, animals were handled daily for three weeks prior the onset of the experiment. All procedures were conducted in accordance with the Guide For The Care And Use Of Laboratory Animals (U.S. Public Health Service, National Institute of Health) and the Institutional Animal Care and Use Committee (IACUC) at the University of Texas at Austin approved the specific protocol for this experiment.

After three weeks of handling, animals were trained to lever press for sugar pellets on a fixed ratio 1 (FR1) schedule for a minimum of 8 days. After the training period, jugular catheterization surgery was performed as previously described (Ikegami and Duvauchelle, 2004).

(+/-) 3,4-methylenedioxymethamphetamine HCl (MDMA) was obtained through NIDA Drug Inventory Supply and Control. For self-administration sessions, MDMA was dissolved in isotonic saline solution (0.9%). The MDMA concentration in solution was adjusted according to the weight of individual animals such that each dose (0.5 or 1.0 mg/kg) was delivered in a volume of 0.1 ml vehicle.

One week after the surgery, rats had access to MDMA (0.5 and 1.0 mg/kg/inj) or saline (control group; 0.1 ml/inj) during 20 daily self-administration sessions. To optimize self-administration behavior (Schenk et al., 2003), MDMA dose/injection was 1.0 mg/kg for Days 1-10 and 0.5 mg/kg for Days 11-20. Two-hr daily sessions were held in operant chambers (28X22X21 cm; Med Associates, St. Albans, VT) equipped with a single retractable operant lever on the right wall with a stimulus light above the lever, and a house light on the opposite wall. Three sets of photocells were evenly spaced on the

front and back walls of the chamber to record locomotor activity. For the first 30 min of each session, the lever was retracted and animals were allowed to habituate to the darkened chamber. At the end of the 30 min period, the house light illuminated and the lever was inserted into the chamber. Each lever-press resulted in the delivery of either MDMA or saline (for control group) injections via an activated syringe pump. Injections were infused over a 6-sec interval, during which time the stimulus light above the lever remained illuminated. The total number of lever presses and activity counts (e.g. number of photobeam breaks) were recorded in 30-min intervals for the duration of the 2-hr session. The experimental programs were controlled and data collected by a Med Pentium 100 MHz computer using Med-PC software.

Rectal temperatures for MDMA and Control groups were monitored each day before and after each operant session using a V911 digital thermometer (Procter & Gamble, Cincinnati, OH), probe inserted to a depth of approximately 4 cm.

**Statistical Analyses**

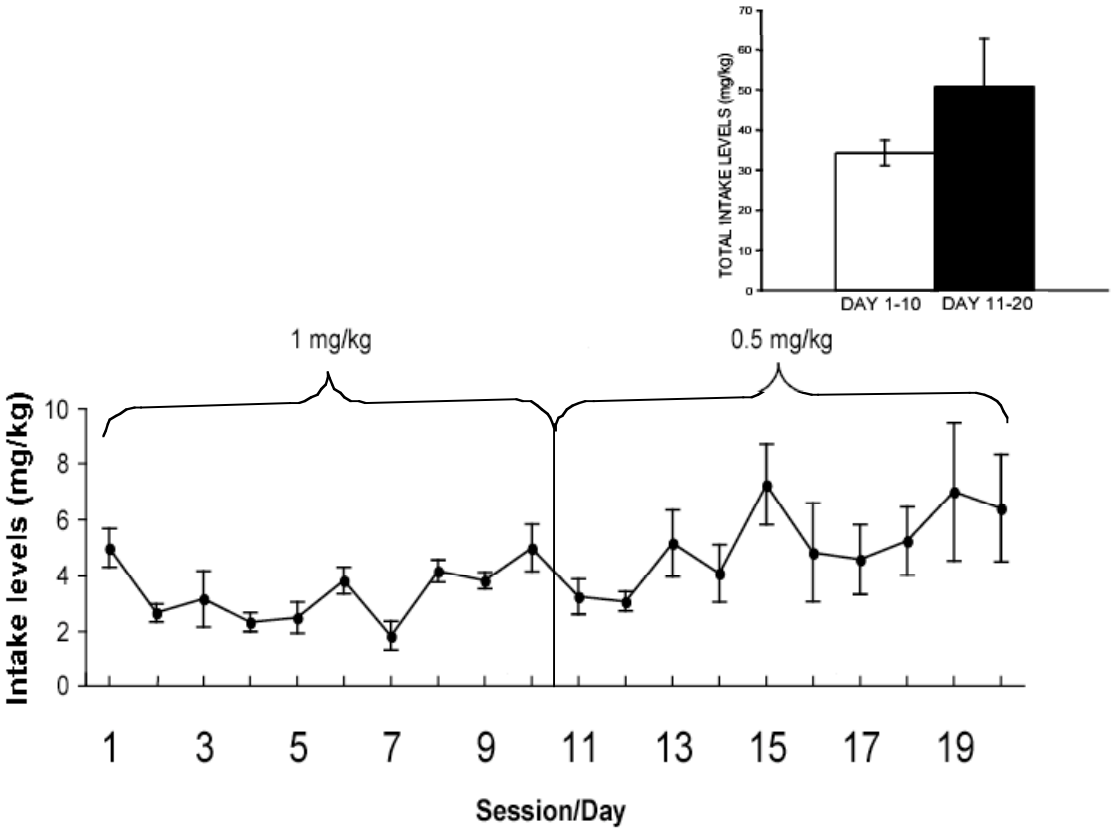
A one-way ANOVA with repeated measures was used to analyze daily MDMA intake (mg/kg) across the 20 sessions. A 2-sample t-test was used to compare cumulative MDMA intake between the two different self-administered MDMA dosages (e.g. total mg/kg for Days 1-10 at 1.0 mg/kg/inj versus Days 11-20 at 0.5 mg/kg/inj). Two-way repeated measures ANOVAs were performed on core temperatures before and after MDMA- and non-reinforced (saline) operant sessions and on locomotor activity measures during sessions. Posthoc tests (Fishers LSD) were performed to determine specific differences when overall ANOVA results indicated significant effects of time or treatment.

## RESULTS

### Daily MDMA intake during self-administration

A two-sample t-test showed that self-administration of 0.5 versus 1.0 mg/kg/inj of MDMA over 10 sessions at each dose did not result in significant differences in overall cumulative intake ($t(5)=1.61$, n.s.; see bar graph insert Fig 1.2). However, a one-way repeated measures ANOVA (MDMA Intake X Day) showed significant Day effects ($F(5,19)=3.07$; $p=0.0002$). Posthoc tests revealed a trend toward higher intake levels during the last few sessions compared to some previous sessions (see Fig 1.2).

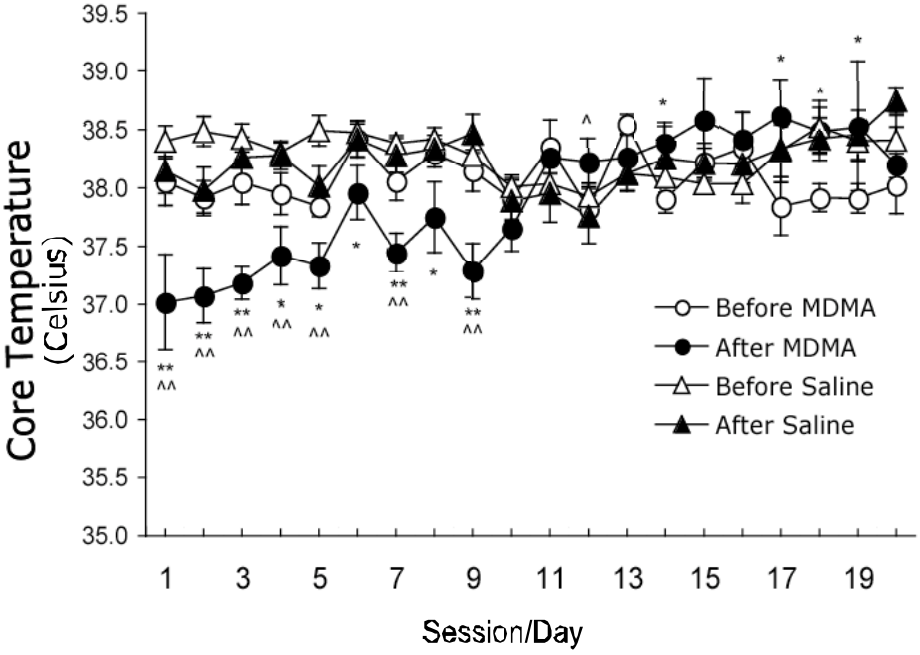
Figure 1.2 Daily MDMA intake levels (mg/kg) during self-administration sessions. Data represent the mean (+/- SEM) across 20 sessions; n=6). Dosage per self-administered injection was 1.0 mg/kg for Days 1-10 and 0.5 mg/kg for Days 11-20. Bar graph insert shows cumulative MDMA intake mean (+/- SEM) for Days 1-10 (white) and 11-20 (black). No significant differences in cumulative intake between dosages were detected by two-sample t-test.



**Core Temperature**

A two-way repeated measures ANOVA (Treatment X Time) on temperatures before and after MDMA and saline self-administration sessions showed significant Time and Interaction effects ($F(19,380)=4.74$ and $F(57,380)=4.3$, $p<0.0001$ for both, respectively), but no significant Treatment effects ($F(3,20)=1.94$, n.s.). Posthoc tests revealed a significant decrease in core temperatures after MDMA self-administration during Days 1-9 compared the same animals before the session and compared to the control group that self-administered saline during operant sessions. From Days 10-20, core temperatures post-MDMA self-administration were no longer hypothermic and progressively increased to significantly higher than pre-MDMA on Days 14, 16-17 and 19 (see Fig. 2.2).

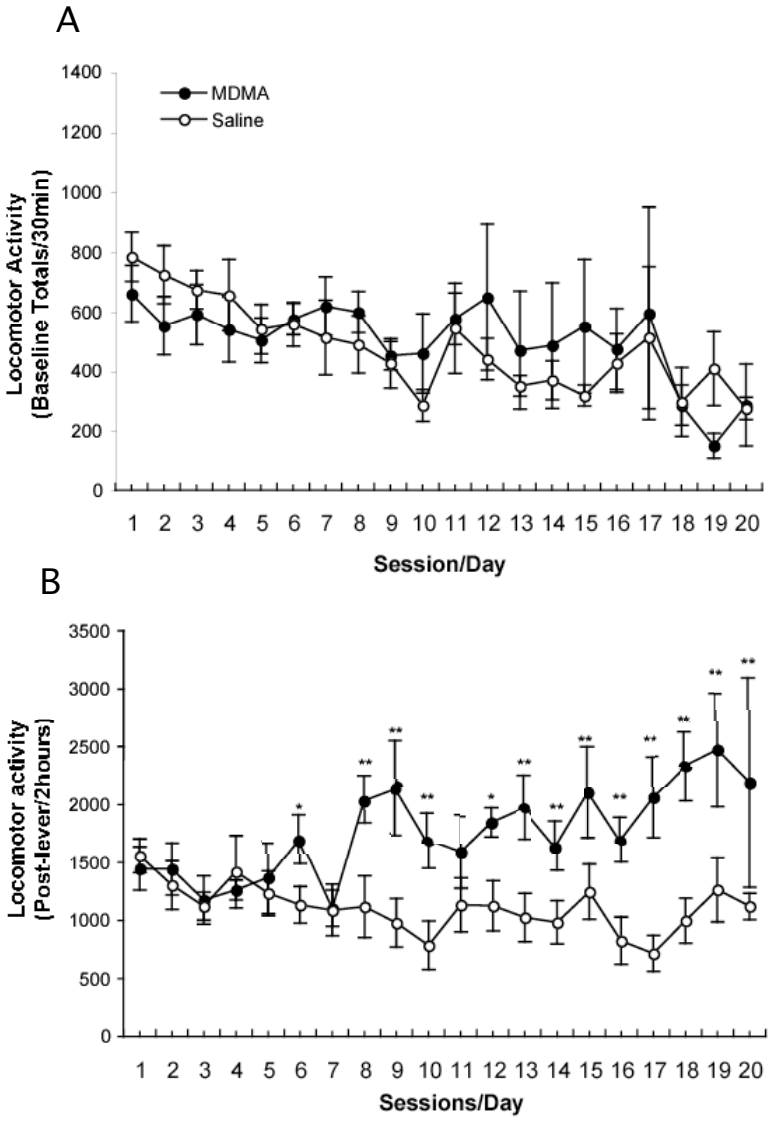
Figure 2.2 Core temperatures before and after MDMA- and non-reinforced operant sessions. Data represents mean (+/- SEM) rectal temperatures. For Days 1-9, temperatures taken after rats self-administered MDMA (n=6) were significantly lower than before sessions, and/or significantly lower than in rats that had self-administered saline (n=6). Between Days 14-20, significantly higher MDMA-induced core temperatures were detected after four sessions. *, ** = significant differences between Before and After MDMA self-administration at $p<0.05$ and 0.01, respectively. ^, ^^ = significant differences between After MDMA and After Saline self-administration sessions at $p<0.05$ and 0.01, respectively (Fisher's LSD).



**Locomotor activity**

A two-way ANOVA on locomotor activity during MDMA and non-reinforced operant sessions showed significant Treatment ($F(1,11)=7.33$; $p=0.02$), Day ($F(19,209) = 1.73$; $p=0.03$) and Treatment X Day interaction effects ($F(19,209)=3.56$; $p<0.0001$). Posthoc tests revealed that locomotor activity was initially equivalent between animals self-administering MDMA and those receiving saline injections. However, MDMA self-administering animals showed significantly increased locomotor activity by Day 8 and maintained higher levels across the remaining sessions with few exceptions (see Fig 3.2).

Figure 3.2 Locomotor Activity during MDMA and saline self-administration sessions. Data represent the mean (+/- SEM, n=6) number of photobeam breaks recorded during A) the habituation period, and B) post-lever activation for every MDMA- and saline-reinforced operant session. Starting at Day 8, locomotor activity during MDMA self-administration was significantly greater than during saline self-administration sessions (with few exceptions). *, ** = significant differences between MDMA and Saline locomotor activity at $p<0.05$ and 0.01, respectively (Fishers LSD).



## DISCUSSION

The present study revealed that MDMA self-administration resulted in experience-dependent changes in body temperature and MDMA-stimulated locomotor activity. Initial hypothermic responses to MDMA gradually reversed to modest hyperthermia, while the lack of locomotor stimulation by MDMA was eventually replaced by hyperlocomotor activation.

In general, MDMA administration in conjunction with ambient temperatures of 22°C or higher are associated with a rise in body temperature (Broening et al., 1995; Dafters, 1994; Nash et al., 1988; O'Shea et al., 2005), while rats kept at cooler temperatures (e.g. 22°C or less) show MDMA-induced hypothermic responses (Dafters, 1994; Dafters and Lynch, 1998; Malberg and Seiden, 1998). Discrepancies regarding whether hypothermia or hyperthermia is produced at 22°C may be attributed to numerous methodologies utilized, including different rat strains, ages, MDMA doses and routes of MDMA administration. In the present study, ambient temperature was set at 22°C and daily room temperature measurements showed temperature variation within 1°C. The current findings are unique in showing experience-dependent fluctuations in body temperature ranging from hypothermia to hyperthermia over the course of long-term MDMA self-administration. These data indicate that even moderate levels of MDMA exposure in the rat influence thermoregulatory processes.

Centrally, endogenous neurotransmitters thought to be involved in controlling body temperature include serotonin (5-HT) and dopamine (DA) (Lee et al., 1985; Schwartz and Erk, 2004; Schwartz et al., 1995). Long-term exposure to MDMA is known to cause neurochemical sensitization in rats, with extracellular DA levels

remaining higher than observed in drug-naïve animals (Kalivas et al., 1998). Hence, it is possible that higher levels of basal DA in drug-experienced animals might activate mechanisms for heat production. Though DA levels were not assessed in the present study, a DA-mediated enhancement of body temperature cannot be ruled out as a possible mechanism for the observed findings.

In agreement with previous reports, the present data demonstrates that MDMA is able to maintain self-administration behavior in rats (Daniela et al., 2004; Schenk et al., 2003) and can produce hyperactivity (Bankson and Cunningham, 2002; Dafters, 1994; Daniela et al., 2004). However, the MDMA-induced increase in activity reported here was not observed initially, but emerged after seven days of MDMA self-administration (see Fig. 3.2), while MDMA intake remained at approximately equivalent levels (see Fig. 1.2). These findings, as observed in prior studies (Dafters, 1995; Green et al., 1995; Kalivas et al., 1998; Modi et al., 2006; Ramos et al., 2004; Spanos and Yamamoto, 1989), indicate the development of MDMA-induced locomotor sensitization. Yet, it is possible that the augmentation of locomotor activity observed here might be associated with the attenuation of hypothermic MDMA effects, and even the subsequent emergence of hyperthermia. In mammals, when body temperature decreases, behavioral mechanisms for heat conservation, such as shivering and increased locomotor activity, are activated (Koteja, 2004). The progressive reduction and reversal of the hypothermic response observed in the present study suggests that compensatory mechanisms of thermogenesis may be triggered in cases of chronic MDMA use. In this regard, elevations in core temperature seen during the last MDMA self-administration sessions may have been the result of increased locomotor activation occurring in response to MDMA-mediated hypothermia.

The ability of moderate MDMA intake to disrupt fundamental homeostatic mechanisms of thermoregulation indicates MDMA's alarming potential for producing biological disorder. Further studies investigating physiological changes induced by self-administered MDMA will help to determine relevant risk factors associated with recreational MDMA use.

## CHAPER 2 SUMMARY

Most drugs of abuse (e.g., cocaine, amphetamine) increase locomotor activity in experimental animals when given acutely. However, the majority of studies assessing increased psychomotor behavior use large doses of the drug. As already mentioned, previous studies have shown low to moderates amounts of MDMA lack locomotor activating properties compared to baseline (Fernandez et al., 2003; Matthews et al., 1989; Spanos and Yamamoto, 1989), yet a dose-dependent increase in activity is observed after acute and repetitive MDMA administration (Callaway et al., 1990; Gold et al., 1988; Slikker et al., 1989; Spanos and Yamamoto, 1989). In addition, changes in core temperature and locomotor activity are also influenced by changes the ambient temperature at which the animals are kept (Broening et al., 1995; Dafters, 1994; Malberg and Seiden, 1998). The results from this study demonstrate moderate amounts of self-administered MDMA did not induce an increase in locomotor activity in naïve animals. The progressive increase in locomotor activity with increase drug intake (locomotor sensitization) is commonly used as a measure of the reinforcing properties of the drug. Our data demonstrate an increase in MDMA-induced locomotor activity compared to saline animals as the self-administration sessions progressed, and that might be indicative of a positive reinforcing action. This work has also shown the initial hypothermic effect of moderate doses of MDMA, and that hypothermia is progressively suppressed with repetitive MDMA exposure.

Although most psychostimulants mediate their actions through increase DA release in various brain regions, the unique pharmacology of MDMA relies in its ability to promote DA and 5-HT release from their nerve terminals. As demonstrated by this study, the proportional increase in extracellular NAcc 5-HT release was higher than that

observed in DA. To date, there is no unanimous consensus on which neurotransmitter (DA or 5-HT) might be implicated in MDMA-associated increase in body temperature. *In vivo* experiments have shown MDMA-induced hyperthermia can be prevented by pre-treatment with a $D_1$ antagonist (Mechan et al., 2002), while various 5-HT receptors antagonist failed to reduce body temperature. On the other hand, a neurotoxic regimen that affects MDMA-induced 5-HT release in the striatum partially prevented MDMA-mediated hyperthermia, but such treatment did not affect DA release (Green et al., 2004; Shankaran and Gudelsky, 1999). Thus, both systems might be involved in MDMA-associated hyperthermic response.

Although the most attention has been concentrated in central DA and 5-HT systems, many of MDMA-associated behavioral and physiological changes might be mediated via peripheral DA, 5-HT, or norepinephrine (NE) $\alpha_1$– and $\alpha_2$–adrenoceptor activations (Bexis and Docherty, 2006; McDaid and Docherty, 2001). In mice, MDMA-induced hyperthermia is initially reversed in $\alpha_2$–adrenoceptor knockout animals (Bexis and Docherty, 2005), and mice lacking UCP3 (a mitochondrial protein found in skeletal muscle) are partially protected against MDMA-induced hyperthermia (Mills et al., 2003). In addition, a recent study (Sprague et al., 2005) has shown MDMA (40 mg/kg s.c) administration induced a profound (35-fold) increase in NE concentration in plasma compared to MDMA-induced DA increase (2.4-fold), while treatment with carvedilol (a $\alpha_2$– and $\beta_2$ –adrenoceptor antagonist) blocked MDMA-associated hyperthermia. Hence, further studies using peripheral 5-HT or DA receptor antagonists, as well as NE $\alpha_2$– and $\beta_2$ –adrenoceptor blockers are needed to elucidate the mechanisms of MDMA-induced disruption of thermoregulation and increased locomotor activity.

# BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF MDMA SELF-ADMINISTRATION


## ABSTRACT

3 ,4-methylenedioxy-N-methamphetamine (MDMA) is a popular psychoactive methamphetamine derivative associated with young adults and all-night dance parties. Though high doses of MDMA have been reported as profoundly neurotoxic in animal studies, the short- and long-term neural effects of MDMA at voluntary intake levels are yet to be determined. In this study, rats self-administered recreational doses of MDMA for a period of 20 days, and *in vivo* nucleus accumbens (NAcc) microdialysis was performed the following day to assess extracellular levels of DA and 5-HT after a single 3.0 mg/kg/intravenous self-administered infusion of MDMA. Daily MDMA self-administration caused a decrease in post-session rectal temperatures from days 1-8. Chronic exposure to MDMA induced behavioral sensitization of locomotor activity responses, and a progressive change in behaviors specific to the 5-HT syndrome (e.g. low body posture, nasal secretions). A significant increase in extracellular NAcc DA and 5-HT were observed in both naïve and experienced animals. However, the magnitud of the DA response was significantly reduced after MDMA self-administration compared to first time users. In addition, MDMA caused proportional greater incrementional changes in 5-HT than in DA compared to baseline, in both naïve and drug-experienced animals. After 7 days of MDMA abstinence, brain neurochemical analysis revealed increased DA levels in the prefrontal cortex (PFC), and NAcc, and reduced NAcc 5-HT levels in MDMA-experienced rats. Hippocampal levels of 5-HT remained unchanged 7 days after treatment in drug experienced animals. In summary, repetitive voluntary MDMA intake

results in neurochemical and behavioral changes that might contribute to MDMA abuse and dependence.

## INTRODUCTION

Psychostimulants, such as MDMA, cocaine, or amphetamine cause behavioral effects via increased DA neurotrasmission in specific brain areas of the mesolimbocortical pathway including the nucleus accumbens (NAcc) and prefrontal cortex (PFC; Ritz and Kuhar, 1993; Yamamoto and Spanos, 1988). These brain areas are part of the "reward" system associated with the incentive motivational properties of drugs of abuse. For example, microdialysis studies in laboratory animals (Carboni et al., 1989; Di Chiara and Imperato, 1988) and imaging studies in humans (Schlaepfer et al., 1997; Volkow et al., 2002) have demonstrated the important role of mesolimbocortical DA in the rewarding effects of many drugs of abuse.

Researchers have investigated the reinforcement efficacy of MDMA using intravenous self-stimulation (Hubner et al., 1988; Lin et al., 1997), conditioned place preference (Bilsky et al., 1991; Marona-Lewicka et al., 1996; Robledo et al., 2004; Schechter, 1991), and self-administration procedures (Beardsley et al., 1986; Fantegrossi et al., 2002; Ratzenboeck et al., 2001; Schenk et al., 2003) in rodents and non-human primates. Although the reinforcement properties of MDMA are lower compared to other drugs of abuse (Fantegrossi et al., 2002; Lile et al., 2005), data indicate MDMA is readily self-administered by drug-naïve animals (Daniela et al., 2004; Schenk et al., 2003).

A prominent effect of MDMA administration is the so-called serotonin behavioral syndrome in laboratory animals, which includes low body posture, forepaw treading, piloerection, exophthalmia, penile erection, ejaculation, salivation, and defecation. These MDMA effects are characteristic of increased 5-HT activity (Green et al., 1995; Hiramatsu et al., 1989). In addition, MDMA causes a dose-dependent increase

in locomotor activity in rodents and non-human primates (Callaway et al., 1990; Gold et al., 1988; Slikker et al., 1989), and the 5-HT system might be partially responsible of this effect. The 5-HT uptake blocker fluoxetine inhibited MDMA-mediated hyperactivity (Callaway et al., 1990; Gold et al., 1989) and RU 24969, a 5-$HT_{1B}$ receptor agonist, causes locomotor hyperactivity in rats, whereas agonists for 5-$HT_{1A}$ and 5-$HT_{2A/2C}$ receptors decrease locomotion (Rempel et al., 1993). However, 5-HT and DA have been shown to influence each others release by interaction with specific DA or 5-HT receptors within the mesolimbocortical pathway (Matsumoto et al., 1996; Porras et al., 2002). The literature suggests both systems play an important role in MDMA-induced locomotor activity and reward, and therefore, both systems should be studied to better understand the neuropharmacology of MDMA.

Long-term alterations in the DA and 5-HT systems after prolonged MDMA exposure might underlie the mechanism for MDMA reinforcement. The purpose of this study was to investigate the acute and chronic physiological, neurochemical, and behavioral changes that occur in rats after MDMA voluntary intake using doses that are closed to those administered by MDMA abusers. Changes in rectal temperature, locomotor activity, and MDMA-induced DA and 5-HT release in the NAcc, and assessments of behaviors associated with MDMA-elicited serotonin syndrome were investigated in drug-naïve and MDMA-experienced rats. The acute and chronic effects of MDMA on total tissue levels of monoamine and metabolites were evaluated in various brain regions.

## MATERIALS AND METHODS

### Animals

Male Sprague-Dawley rats (4 weeks, Charles River Laboratories, Inc. Wilmington, MA) were housed in a room with controlled temperature (22±1°C) and reverse light cycle (lights off 7:00am-7:00pm). Animals were kept in clear cages and water and laboratory food pellets were available *ad libitum*. To minimize stress, animals were handled daily for two weeks prior the onset of the experiment. Two weeks after the animals arrive to the laboratory, they were trained to lever press for sugar pellets on a fixed ratio 1 schedule for a minimum of 7 days (10 min/day). All procedures were conducted in accordance with the Guide For The Care And Use Of Laboratory Animals (U.S. Public Health Service, National Institute of Health).

### Surgeries

After the training period, jugular catheterization and stereotaxic surgeries were performed under general anesthesia (sodium pentothal (50mg/kg, i.p.) and supplemental chloral hydrate (80mg/kg, i.p.) as needed to prolong anesthesia. Atropine sulfate (250 μg) was injected subcutaneously to prevent excessive respiratory secretions. The catheter tip was introduced into the right external jugular vein and advanced towards the heart using a fine pair of forceps. A midline incision was made with a surgical scalpel in the skin overlying the skull. The free edges of the skin were retracted, and a twist hole was drilled at the following flat skull coordinates: 2.0mm anterior to bregma, and 1.2 mm lateral to the midline. A guide cannula (Plastic One, Roanoke, VA) was positioned in this hole and lowered 2.5mm ventral to the dural surface. Four other holes were made around the cannula to bear anchor screws. The free end of the catheter was tunneled through the

subcutaneous tissue around the shoulder and craneoplastic cement (Plastic One, Roanoke, VA) was distributed around the cannula, catheter and supporting screws. A dummy cannula (Plastic One, Roanoke, VA) was inserted inside the guide cannula to prevent debris build-up within the lumen.

**Dialysis prove and *in vitro* probe recovery analysis**

Microdialysis probes were constructed using polyethylene 20 tubing as the inlet and fused silica tubing (75 $\mu$m i.d.) inserted into a 2.5mm section of cellulose (13 kDalton cut-off; Spectrum, Houston) fitted through a 26 gauge internal cannula (Plastic One, Roanoke, VA). The outlet consisted of fused silica coated with polyethylene 50 tubing. To determine recovery rates for each probe, Hamilton syringes were filled with freshly prepared filtered artificial cerebral spinal fluid (ACSF) solution, and pumped continuously through the probe at a rate of 1.63 $\mu$l/min. Probes were placed in a beaker containing ascorbate (1%), 4nM DA, and 4nM 5-HT maintained at 37°C. 10-min dialysis samples from each probe were collected, and assayed by high performance liquid chromatography (HPLC) coupled to amperometric detection (Shizeido Capcell, 1.5 mm X 50 mm, 3 $\mu$m particle size column, ESA 5041 amperometric cell detector with the oxidizing potential set to +200 mV, sensitivity 100 pA, guard cell potential 400 mV; ESA, Inc., Chelmsford, MA). The mobile phase contained 150 mM Na2H2PO4, 50 $\mu$M EDTA disodium salt, 4.76 mM citric acid, 4.5–6.0 mM sodium dodecyl sulfate, 12.5% (v/v) acetonitrile, 12.5% (v/v) methanol, pH 5.6, constant flow rate of 0.2 ml/min. Probe recovery was calculated by comparing the peak heights of each dialysate and those from a 25% recovery standard solution. Average DA and 5-HT recoveries were ~ 13-15%. The limit of detection (at S/N ratio 3) was 0.36 fg/sample for DA, and 0.38 fg/sample for 5-HT. Data was collected and analyzed using an ESA Model 500 Data station.

**Experimental groups**

One week after the surgery, animals were randomly assigned to one of four experimental groups: Experienced MDMA, experienced saline, naïve MDMA, and naïve saline. Experienced MDMA rats underwent ten days (two hours a day) acquisition (+/-)-MDMA sessions (1mg/kg infusions, delivered in 0.1ml over approximately 6 s.), plus ten more days (+/-)-MDMA sessions (0.5mg/kg infusions). Experienced saline animals self-administer saline (non-reward) infusions under the same operant conditions. Naïve MDMA and naïve saline animals did not undergo acquisition sessions.

Acquisition sessions were conducted in operant chambers (28X22X21 cm, Med Associates, St. Albans, VT). A single retractable operant lever was located on the right wall, and each lever-press was accompanied by illumination of a stimulus light located above the lever. Inside the chamber, a swivel was attached at one end by Tygon tubing to a syringe mounted on a motor-driven syringe pump (Razel, St. Albans, VT) located outside the chamber. At the other end of the swivel a cannula connector with spring (Plastic One, Roanoke, VA) was attached to the animal's catheter. A house light was located at the top of the left metal wall. During daily acquisition sessions, animals had an initial 30-min habituation period with the house light off and the lever retracted, followed by 2-hour self-administration sessions when the lever became available. After each injection, a 20 s time-out occurred during which time the lever was retracted and no infusions could be delivered. Locomotor activity counts were daily recorded during the basal conditions and for the first 30 min interval post lever activation. Rectal temperatures of MDMA animals were monitored each day before and after each operant session using a V911 digital thermometer (Procter & Gamble, Cincinnati, OH), probe inserted to a depth of approximately 4 cm.

Total number of lever presses were assessed during the 2-hours self-administration sessions for MDMA and Saline animals. During MDMA self-administration sessions, animals were videotaped during three 1-minute intervals. Saline animals were videotaped on days 1, 11, and 20 to provide non-drug behavioral comparisons. The following behaviors were scored by "blind" observers: a) low body posture, b) reciprocal forepaw treading (RFT; rhythmic dorsal—ventral movements of forelimbs, with no net movement of hindlimbs), c) piloerection d) snout porphyrin-enriched secretions, and e) continuous sniffing (lasting more than 10 seconds).

After the last session, animals were implanted with a microdialysis probe into the Nacc. *In vivo* microdialysis test sessions were conducted 24 hr later to assess NAcc DA and 5-HT levels after a single self-administered MDMA injection (3 mg/kg) in experienced and naïve MDMA animals. In addition, experienced and naïve saline animals were tested after a single non-reinforced (saline) lever response.

***In vivo* microdialysis test sessions**

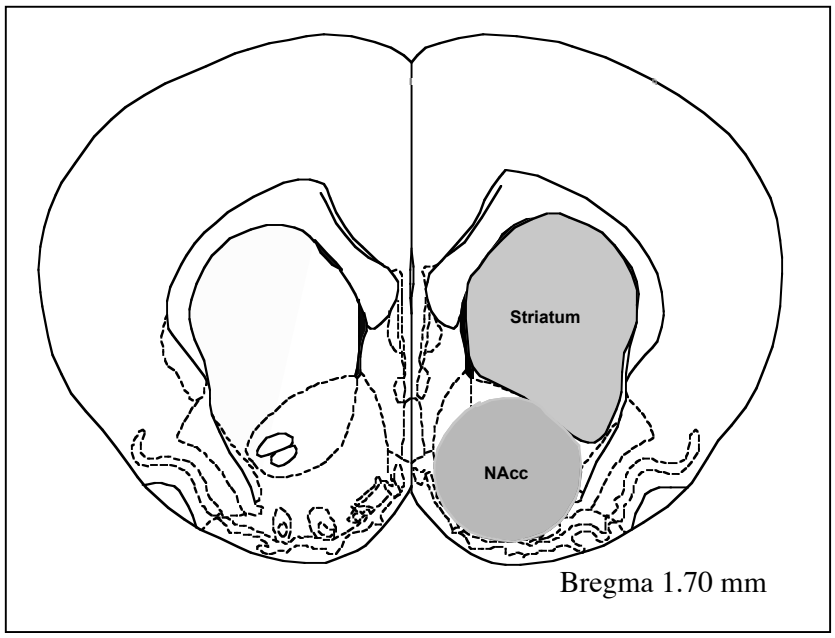
Rats were briefly anesthetized with 1.5% isoflurane while the microdialysis probe was lowered through the indwelling cannula. Artificial CSF was then pumped through the probe at a rate of 0.2 $\mu$l/min for approximately 12 hours. The pump speed was then increased to 1.65 $\mu$l/min, and one hour later, the first 10 min basal sample was collected. A total of three 10-min basal samples were collected before treatment and 6 more samples after the infusion. Locomotor activity counts were recorded for the entire 1.5-hour session. Dialysate DA and 5-HT content were assessed using HPLC coupled to amperimetric detection (as described above).

**Brain tissue analyses**

Rats were euthanized 24h or 7 days after the last MDMA injection (dialysis test day), and their brains were quickly removed and placed on an ice-chilled plate on their ventral surface. Dissections were free-hand and specific regions contralateral to the dialysis probe location were isolated in the following manner: the olfactory bulbs and portions of the olfactory tubercle were removed and a 1 mm transverse cut was made using a razor blade through the right frontal lobe to retrieve the frontal cortex sample. A second transverse cut was made 2 mm posterior to the first cut and the isolated block of tissue was placed rostral surface down. The ventromedial portion of the nucleus accumbens was isolated with a 2.5 mm biopsy punch using the anterior commissure as a landmark. Dorsal and lateral cortex aspects were trimmed from the remaining piece of tissue to obtain the anterodorsal striatum. A mid-sagittal cut was made with the razor blade along the longitudinal fissure. The dorsal cortex was peeled downward with the help of a blunt spatula to reveal the hippocampus. The most dorsal-anterior aspect of the hippocampus was then trimmed of dorsal fornix and pulled free using fine dissection forceps (see Diagram pg. 67)

Drawings of coronal A) and sagittal B) rat brain sections illustrating brain regions isolated for monoamine and metabolites HPLC-EC analysis

A

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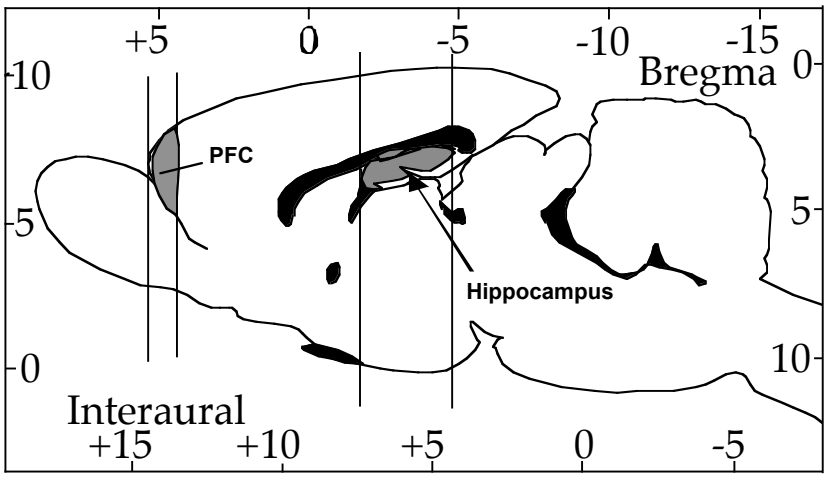
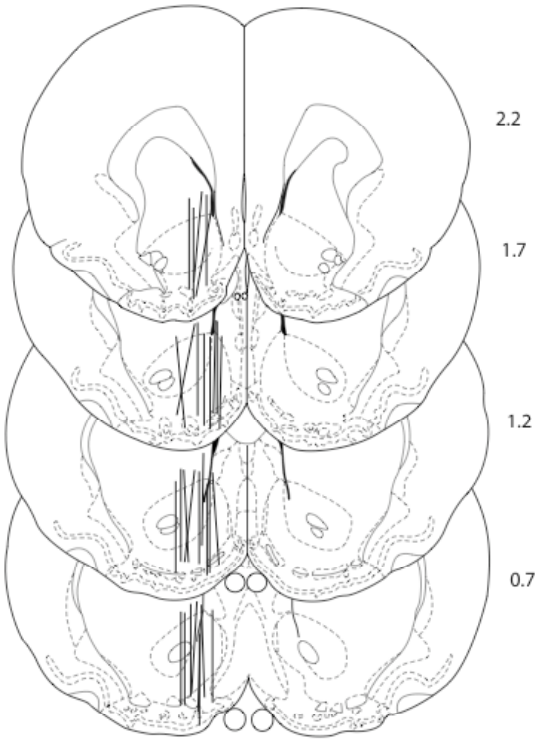


Tissue samples were briefly kept on dry ice and later stored at -80°C until subsequent analysis. For monoamine and metabolites detection, samples were sonicated in 0.1 M perchloric acid containing 347 $\mu$M sodium bisulphite, and 134 $\mu$M ethylenediaminetetraacetic acid (EDTA) disodium salt. Homogenates were centrifuged at 16,000 x g for 20 min and the supernatant removed, centrifuged again, and analyzed for levels of DA, DOPAC, HVA, 5-HT and 5-HIAA by high performance liquid chromatography with an electrochemical coulometric array detector (HPLC-EC). Monoamines were measured at +150mV and +300mV. The mobile phase consisted of 4 mM citric acid monohydrate, 8 mM ammonium acetate, 54 mM EDTA disodium salt, 230 mM 1-octanesulfonic acid sodium salt monohydrate (final pH 2.5) and 5% methanol. The concentrations of neurotransmitters and metabolites were determined by comparison with calibration curves prepared from authentic monoamine standards (Sigma-Aldrich Corp., St. Louis, MO). Striatal, PFC, hippocampal, and NAcc samples of animals that self-administered saline were analyzed as well.

**Histology**

After completion of the experiment, animals were euthanized, and microdialysis probes placement into the NAcc confirmed with histological analyses of 60 $\mu$m coronal sections stained with cresyl violet. Figure 1.3 shows a representative diagram of the microdialysis probe placements in coronal sections of the rat brain.

Figure 1.3 Schematic representation of the active dialysis probe membrane region in the NAC of MDMA and saline treated animals (n=30) that completed the dialysis experiment. Numbers depicted next to each brain slice indicate the mm anterior to bregma. The diagram was drawn with the assistance of the atlas of Paxinos and Watson (Paxinos and Watson, 1997).



### Statistical analyses

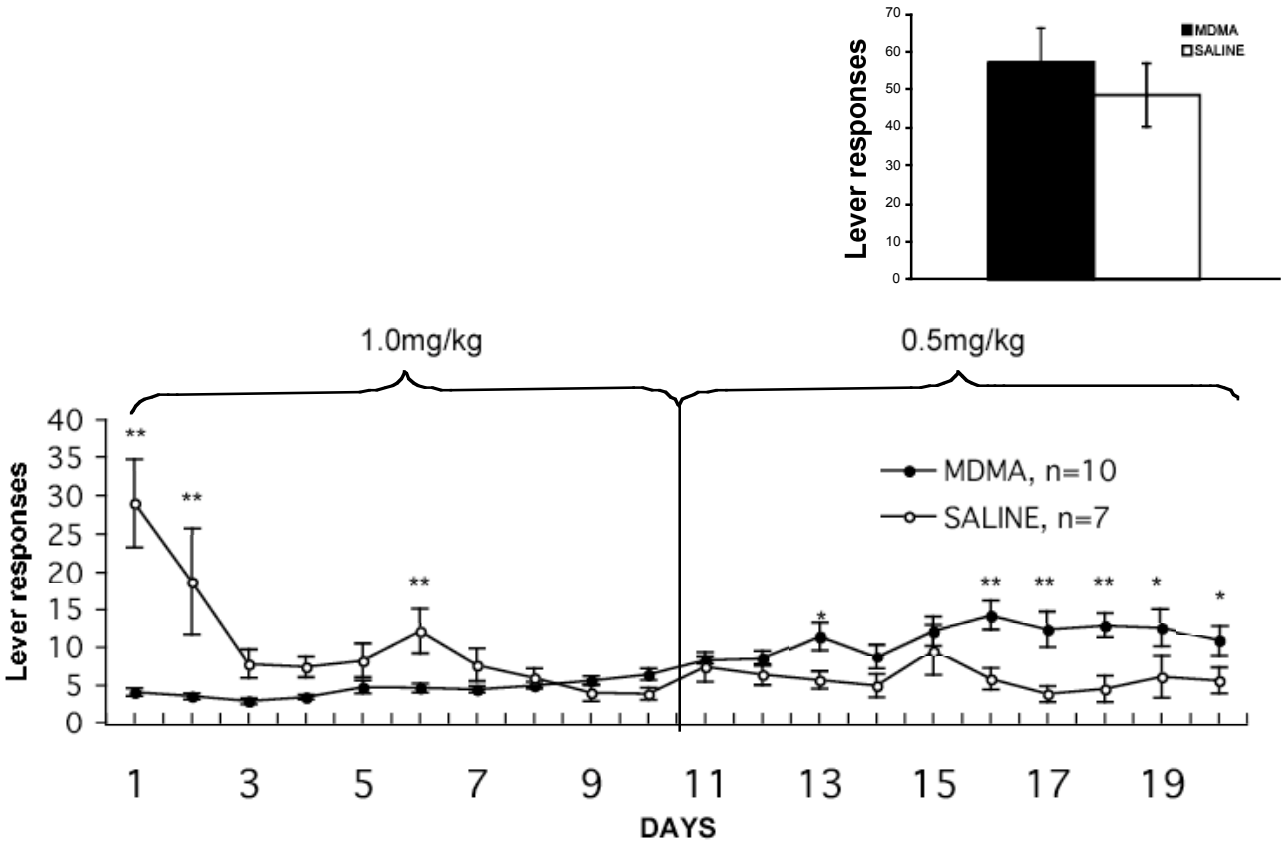
The data are presented as mean ± SEM values. Two-way repeated measures ANOVAs were used to analyze lever presses, rectal temperatures, and locomotor activity during self-administration sessions, as well as locomotor activity and DA and 5-HT levels during microdialysis test sessions. Posthoc tests (Fishers LSD) were performed to determine specific group differences when overall ANOVA results showed significant time or treatment differences. Two-tailed student's t tests were used to analyze DA, DOPAC, HVA, 5-HT and 5-HIAA tissue contents as compared to their respective saline controls.

## RESULTS

### MDMA self-administration/Lever responses

The results of the two-way repeated measures ANOVA test show a significant time effect ($F(19,285)=5.07$; $p<0.0001$), and group X time interaction ($F(19,285)=11.75$; $p<0.0001$). As previously shown (Schenk et al., 2003), while no significant effect of group was observed ($F(1,15)=0.04$; $p=0.834$). Posthoc tests reveal dose-dependent changes in response with animals pressing significantly more when MDMA dose was lowered. In addition, control animals initially showed a greater number of lever responses compared to the (+/-)-MDMA group on days 1, 2, and 6, but as sessions progressed this pattern was reversed.

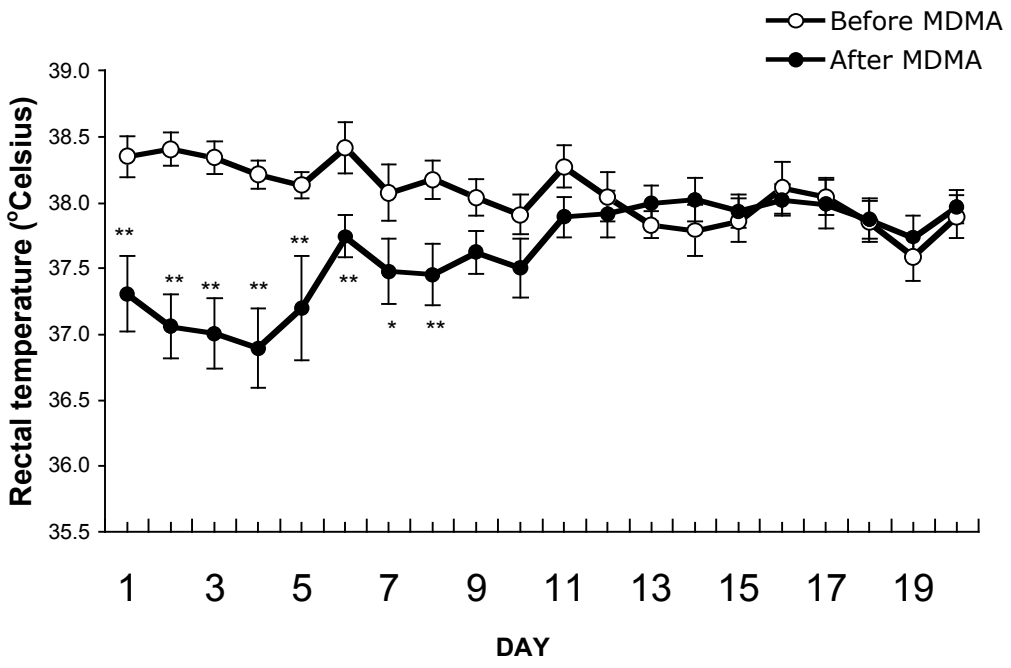
Figure 2.3 Total number of lever responses during (+/-)-MDMA and non-reinforced (saline) self-administration sessions. Data represent the mean (+/- SEM) number of lever presses during the course of 20 daily sessions. *, ** = sig difference at $p<0.05$, $p<0.01$ between same-day MDMA and non-reinforced lever responses. Insert shows the average number of lever responses (± SEM) recorded the last day of food training during a 10 min interval in both experienced-MDMA and saline rats.



**(+/-)-MDMA self-administration/Core temperature**

A two-way repeated measures ANOVA (treatment X time) on temperatures before and after (+/-)-MDMA sessions showed significant time ($F(19,342)=1.77$, $p=0.0243$), and treatment X time ($F(19,342)=6.60$, $p<0.0001$) interaction effects. Post hoc tests revealed a significant decrease in core temperatures after (+/-)-MDMA self-administration during Days 1-8 (see Fig. 3.3) compared the same animals before the session.

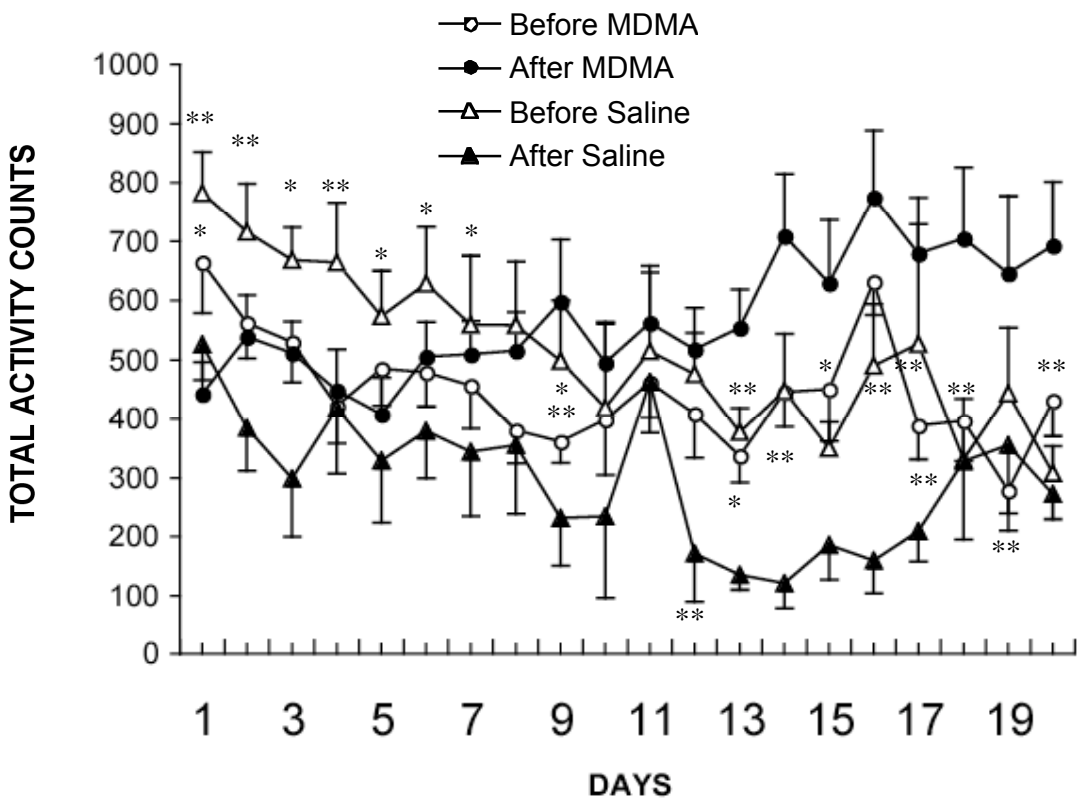
Figure 3.3 Effect of (+/-)-MDMA daily self-administration on rectal temperatures. Data represent the mean (+/- SEM, n=10) rectal temperature, before and after each session, during the course of 20 days. *, ** = sig difference at $p<0.05$, $p<0.01$ between same-day temperatures.



**(+/-)-MDMA self-administration/Locomotor activity**

Locomotor activity was measured as number of photobeam interuptions during 30 min intervals in saline and MDMA animals. Fig 4.3 shows locomotor activity counts recorded 30 min before (+/-)-MDMA or saline availability and during the first 30 min of MDMA or saline self-administration. A two-way repeated measures ANOVA (group X day) on locomotor activity before and after (+/-)-MDMA and saline sessions showed significant group ($F(3,30)=5.24$, $p=0.005$), day ($F(19,570)=2.89$, $p<0.0001$), and group X day ($F(57,570)=2.37$, $p<0.0001$) interaction effects. Post-hoc tests show singnificant increase after MDMA availability for day 9 ($p<0.01$), 13 ($p<0.05$), 14 ($p<0.01$), day 15 ($p<0.05$), and days 17-20 ($p<0.01$). In addition, a significant decrease in locomotor activity was detected post-lever activation in saline animal for most sessions.

Figure 4.3 Locomotor Activity during MDMA and saline self-administration sessions. Data represent the mean (+/- SEM) total activity counts recorded during baseline (30 min) and the first 30-min interval post lever activation. *, ** = significant differences between basal and post-lever activation locomotor activity at $p<0.05$ and 0.01, respectively (Fishers LSD).



**Effect of repetitive (+/-)-MDMA self-administration on 5-HT syndrome specific behaviors**

Fig 5.3 illustrates the effect of daily (+/-)-MDMA self-administration on specific behaviors of the 5-HT syndrome. Data are presented as total number of animals that displayed A) low body posture, B) snout porphyrin-enriched secretions, C) continuous sniffing, D) piloerection, and E) RFT for the 20 days self-administration sessions. In addition, saline animals are shown for days 1, 11, and 20 for comparison. (+/-)-MDMA self-administration induced a progressive increase in the number of animals displaying secretions, and a progressive decrease in the number of animals showing low body posture behavior. Reciprocal forepaw treading was the least observed behavioral component. Control animals did not show low body posture, continuous sniffing, or RFT.

Figure 5.3 Comparison between specific serotonin syndrome behaviors displayed daily in animals that self-administered MDMA (n=9). The ordinate depicts the total number of animals that showed A) low body posture, B) porphyrin secretions, C) continuous sniffing, D) piloerection, and E) RFT at each daily MDMA self-administration session. Behaviors observed on days 1, 11, and 20 during saline self-administration sessions were also included.

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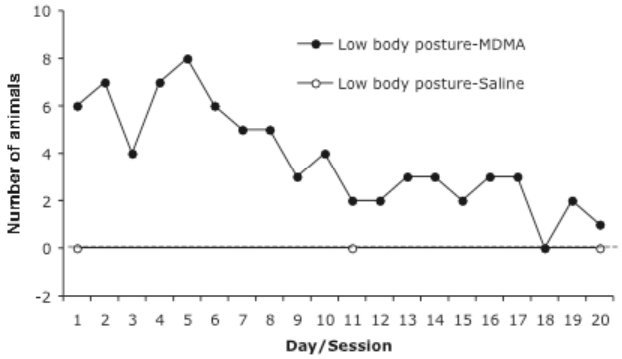


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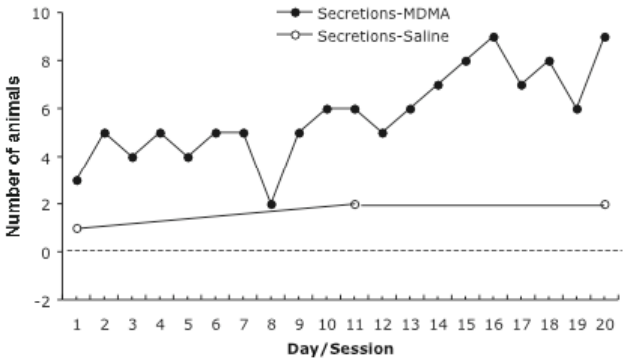


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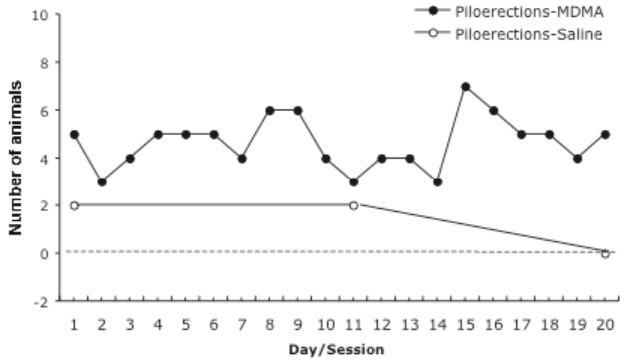

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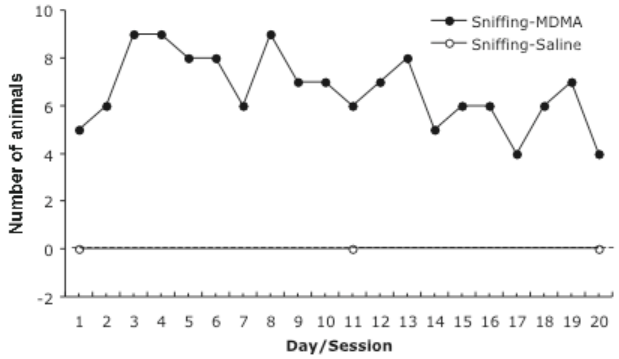
Sniffing-MDMA
Sniffing-Saline
Number of animals
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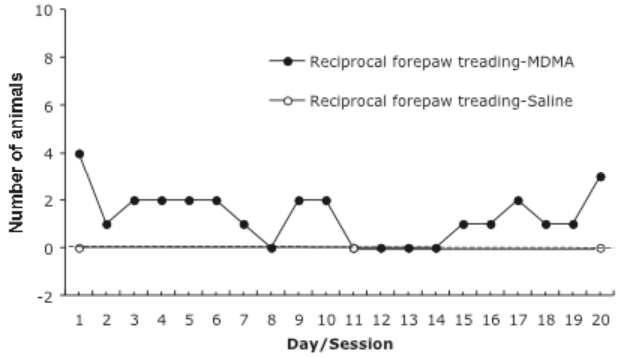
6
4
2
0
-2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Day/Session

E


Reciprocal forepaw treading-MDMA
Reciprocal forepaw treading-Saline
Number of animals
10
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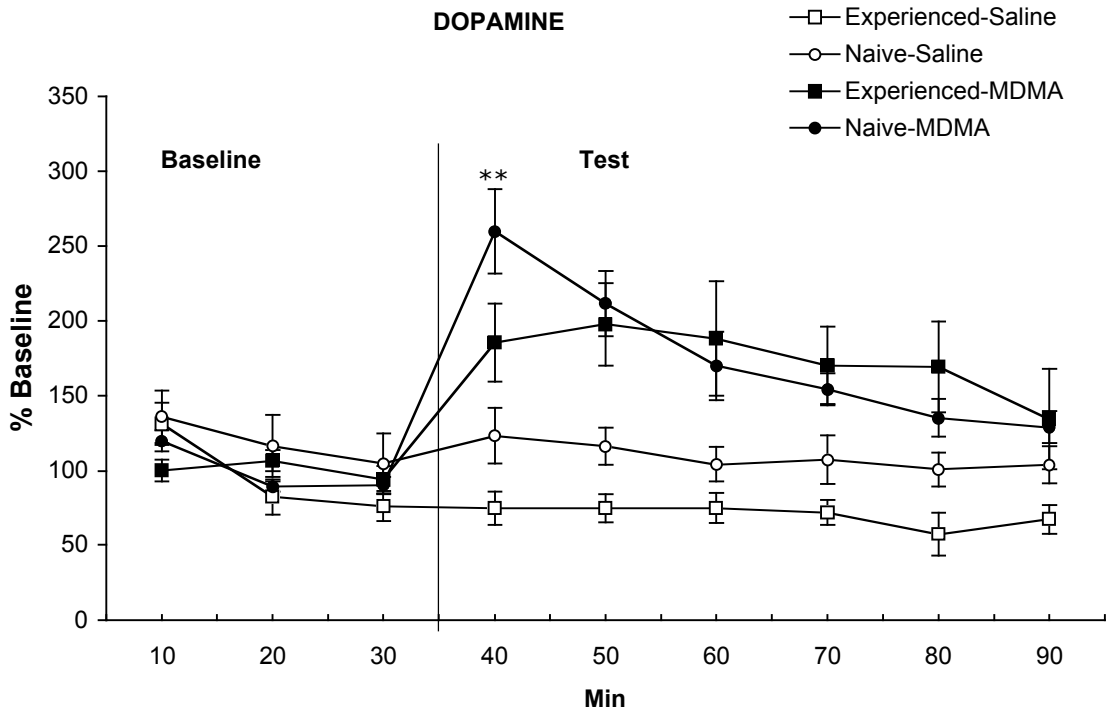
Day/Session

**Effect of a single (+/-)-MDMA (3mg/kg) or saline self-administered infusion on dialysate DA and 5-HT in the NAcc in drug-experienced and naïve animals**

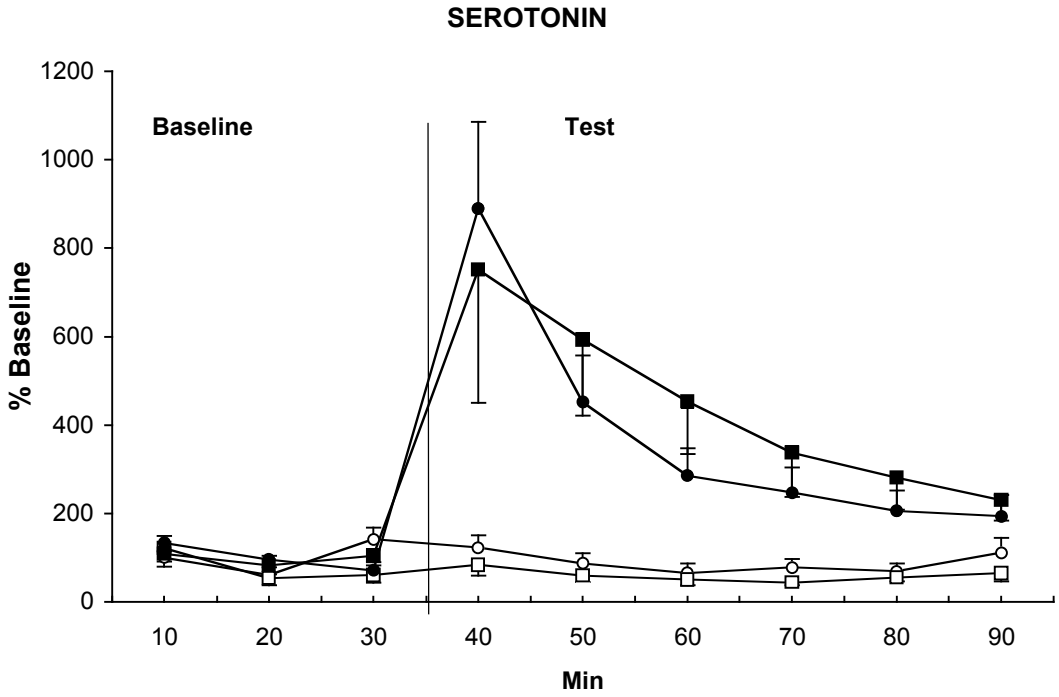
Figure 6.3 (A and B) show the effect of a single self-administered infusion of (+/-)-MDMA (3mg/kg, i.v.) on accumbal extracellular levels of DA and 5-HT (expressed as percent of baseline). Analysis with two-way ANOVA repeated measures indicated a significant time ($F(8,208)=6.87$; $p<0.0001$), group ($F(3,26)=5.20$; $p=0.006$), and time X group interaction ($F(24,208)=2.48$; $p=0.0003$) with respect to basal extracellular DA levels. Post-hoc comparison, with Fisher's LSD test, revealed (+/-)-MDMA (3mg/kg, i.v.) increased DA levels in naïve (at 10, 20, and 30 min post infusion) and experienced animals (at 10, 20, 30, and 40 min post infusion) compared to baseline. Extracellular DA levels in saline naïve and saline experienced animals remained unchanged after a non-rewarding saline infusion. There were also significant differences in DA levels (at time points 10 and 50 post saline infusion) between naïve and experienced saline rats. Similarly, analysis with repeated measure two-way ANOVA indicated a significant time ($F(8,208)=15.94$; $p<0.0001$), group ($F(3,26)=6.82$; $p=0.0015$), and time X group interaction ($F(24,208)=5.47$; $p<0.0001$) with respect to basal extracellular 5-HT levels. (+/-)-MDMA (3mg/kg, i.v.) dramatically increased accumbal 5-HT during the first 20-min after the drug infusion in drug-naïve, and for 40 min in experienced (+/-)-MDMA rats. (+/-)-MDMA evoked 5-HT release was more potent (approximately 800% of basal) than DA release in both groups of animals. In addition, post-hoc comparison indicated NAcc DA release was more pronounced on naïve-animals than on (+/-)-MDMA chronic animals at time point 10 min post infusion ($p<0.01$; see Fig 6.3A).

Figure 6.3 Effect of MDMA (3mg/kg, i.v.), or saline infusion on extracellular NAcc DA and 5-HT levels. Panel A shows the mean extracellular dopamine content (+SEM) expressed as % baseline during three 10-min basal samples and during six 10-min samples post drug infusion in: Saline naïve animals (n=9) and Saline experienced animals (n=5) after a non-rewarding (saline) infusion, and MDMA naïve animals (n=10) and MDMA experienced animals (n=6) after a 3mg/kg MDMA infusion. Panel B shows the data for extracellular 5-HT content expressed as % baseline for the same animals during the same experimental conditions. The means (±SEM) of basal extracellular DA levels (expressed as pg/µl corrected according to probe recovery) were as follows: 0.597±0.07, 0.449±0.09, 0.401±0.14, and 0.450±0.08 in naïve saline, experienced saline, naïve MDMA and experienced MDMA rats, respectively. The means (±SEM) of basal extracellular 5-HT levels (expressed as pg/µl corrected according to probe recovery) were 0.219±0.04, 0.114±0.02, 0.196±0.06, and 0.091±0.03 in naïve saline, experienced saline, naïve MDMA and experienced MDMA rats, respectively. ** = significant differences between naïve and experienced MDMA animals at $p < 0.01$ (Fishers LSD).

A



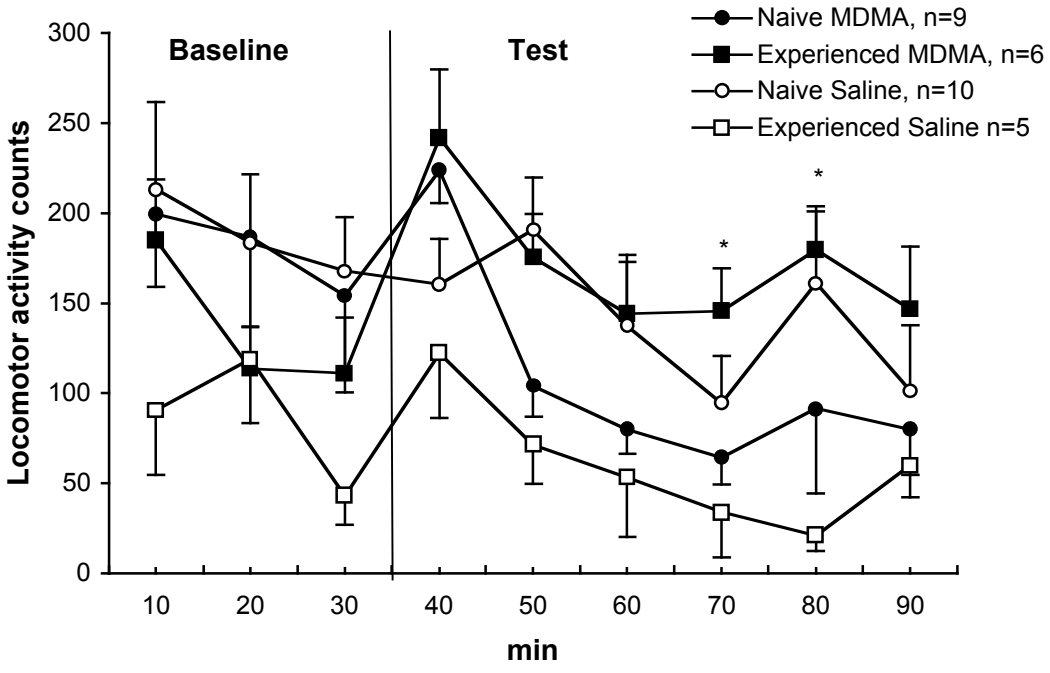
B


SEROTONIN
Baseline
Test
% Baseline
1200
1000
800
600
400
200
0
10
20
30
40
50
60
70
80
90
Min

**Effect of single (+/-)-MDMA (3mg/kg) or saline self-administered infusion on locomotor activity in drug-experienced and naïve animals**

Fig 7.3 shows the effect of a single 3mg/kg (+/-)-MDMA infusion on locomotor activity in drug naïve and experienced animals, and the effect of a non-rewarding infusion in saline naïve and saline experienced animals, respectively. Data are represented as activity counts during 10 minutes intervals for the 30-min habituation period, and during the 60-min period post infusion for each treatment group. Analysis with two-way ANOVA repeated measures indicated a significant treatment ($F(3,26)=4.71$; $p=0.0093$), and time effect ($F(8,208)=4.63$; $p<0.0001$). Post hoc analysis showed a 3mg/kg (+/-)-MDMA challenge had different effects on locomotor activity in naive and drug-experienced animals in that (+/-)-MDMA only induced an increase in activity in MDMA-experienced animals (at 10 min post (+/-)-MDMA infusion compared to baseline activity during the last 20 min; $p<0.01$).

Figure 7.3 Effect of self-administered MDMA or saline on locomotor activity during microdialysis test sessions. Locomotor activity was recorded in 10 min intervals during the habituation period (30min), and the first 60 minutes post MDMA (3mg/kg) infusion in MDMA naïve (n=9), and experienced animals (n=6), and post saline infusion in saline naïve (n=10) and experienced rats (n=5). Data are illustrated as mean activity counts (± SEM). * = sig difference at $p<0.05$ between pre and post-treatment activity.



**Effect of (+/-)-MDMA self-administration on tissue monoamines and metabolites content**

The contralateral hemisphere of the brain was used to assess monoamine and metabolites tissue content by HPLC. Neurochemical tissue analysis revealed monoamine levels, although not significantly, were lower 24 hours after the microdialysis test, in the NAcc and striatum compared to sham operated animals. These results suggest lower levels are the results of microdialysis test conducted in the ipsilateral hemisphere (from which monoamines were collected), and are not at odd since the extracellular space is suspended in cerebrospinal fluid that circulates between ventricles. Interestingly, DA levels were slightly increased in the PFC of animals that underwent microdialysis compared to sham-operated animals. Figure 8.3 shows the acute and long-term effect of (+/-)-MDMA self-administration on 5-HT and DA brain tissue content, in drug naïve and experienced animals. Animals were euthanized 24 hours, or 7 days, after the last self-administration session (microdialysis test) and individual Student's t tests revealed hippocampal levels of 5-HT (in pg/mg tissue) were significantly reduced in naïve animals 24 hours and 7 days, and in experienced rats 24 hours post-treatment (see Fig. 8.3, panel A). (+/-)-MDMA self-administration also caused short-term reductions in NAcc 5-HT in MDMA naïve rats, and long-lasting reductions in experienced animals (see Fig. 8.3, panel B). Seven days after (+/-)-MDMA self-administration, concentrations of DA were increased in both naïve and experienced animals in the NAcc (panel C). Striatal levels of 5-HT and DA were increased in naïve animals one week after a single 3mg/kg (+/-)-MDMA infusion (panel D and E, $p<0.05$). Levels of 5-HT were decreased in the PFC of naïve animals 24 hours and 7 days after microdialysis (panel F). In addition, chronic (+/-)-MDMA self-administration resulted in long-lasting increase in DA levels in the PFC.

Figure 8.3 Short and long-term effect of MDMA self-administration on brain tissue DA, and 5-HT.

**Panel A** shows hippocampal 5-HT tissue content (expressed as pg/mg wet tissue) 24 hours and 7 days after the microdialysis test session in drug naïve and experienced animals. Each value represents the mean (± SEM) for naïve saline/24 hours (n=7), naive MDMA/24h (n=12), naïve saline/7days (n=5), naive MDMA/7days (n=9), experienced saline/24 hours (n=6), experienced MDMA/24h (n=7), experienced saline/7days (n=5), and experienced MDMA/7days (n=8).

**Panel B** shows NAcc 5-HT tissue content for naïve saline/24 hours (n=7), naive MDMA/24h (n=11), naïve saline/7days (n=5), naive MDMA/7days (n=5), experienced saline/24 hours (n=6), experienced MDMA/24h (n=8), experienced saline/7days (n=5), and experienced MDMA/7days (n=7).

**Panel C** shows NAcc DA tissue content for naïve saline/24 hours (n=7), naive MDMA/24h (n=10), naïve saline/7days (n=5), naive MDMA/7days (n=5), experienced saline/24 hours (n=6), experienced MDMA/24h (n=8), experienced saline/7days (n=5), and experienced MDMA/7days (n=7).

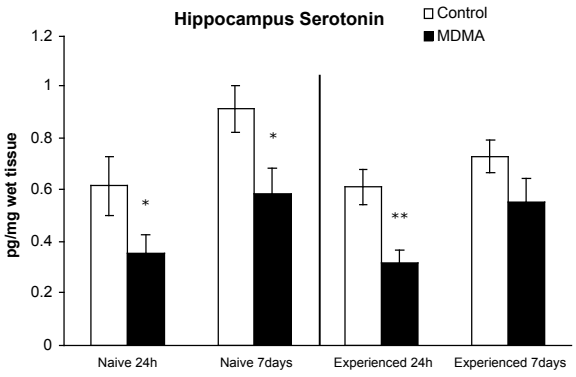
**Panel D** shows striatal 5-HT tissue content for naïve saline/24 hours (n=7), naive MDMA/24h (n=11), naïve saline/7days (n=5), naive MDMA/7days (n=9), experienced saline/24 hours (n=6), experienced MDMA/24h (n=8), experienced saline/7days (n=7), and experienced MDMA/7days (n=8).

**Panel E** shows striatal DA tissue content for naïve saline/24 hours (n=7), naive MDMA/24h (n=10), naïve saline/7days (n=5), naive MDMA/7days (n=7), experienced saline/24 hours (n=6), experienced MDMA/24h (n=8), experienced saline/7days (n=7), and experienced MDMA/7days (n=8).

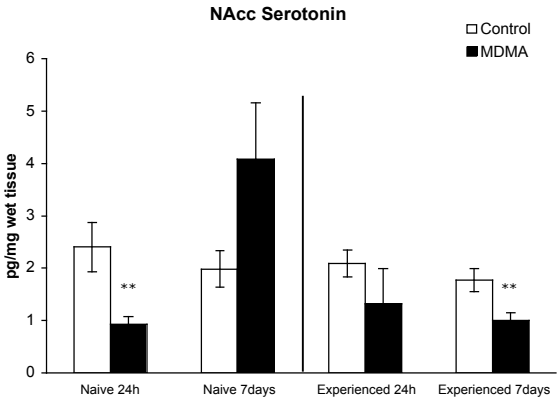
**Panel F** shows prefrontal cortex 5-HT tissue content for naïve saline/24 hours (n=6), naive MDMA/24h (n=12), naïve saline/7days (n=5), naive MDMA/7days (n=8), experienced saline/24 hours (n=6), experienced MDMA/24h (n=8), experienced saline/7days (n=7), and experienced MDMA/7days (n=10).

**Panel G** shows prefrontal cortex DA tissue content for naïve saline/24 hours (n=6), naive MDMA/24h (n=12), naïve saline/7days (n=5), naive MDMA/7days (n=8), experienced saline/24 hours (n=6), experienced MDMA/24h (n=8), experienced saline/7days (n=7), and experienced MDMA/7days (n=10). *, ** = significant differences between control and MDMA groups at $p<0.05$ and 0.01, respectively (Student's t test).

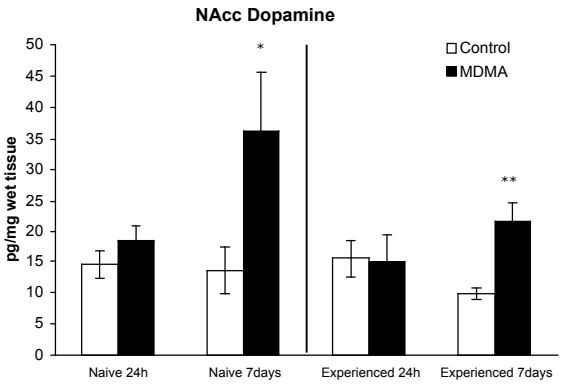
A


Hippocampus Serotonin
Control
MDMA
pg/mg wet tissue
1.2
1
0.8
0.6
0.4
0.2
0
*
*
**
Naive 24h
Naive 7days
Experienced 24h
Experienced 7days

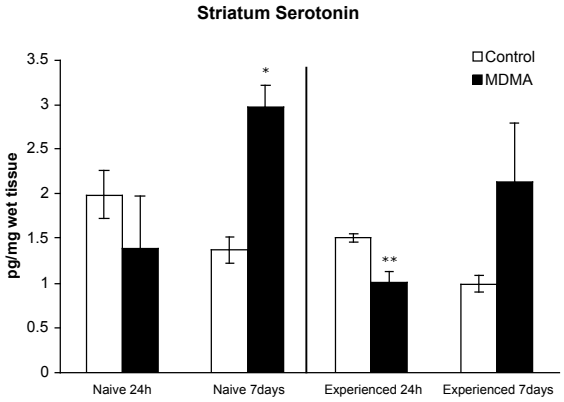
B


NAcc Serotonin
Control
MDMA
pg/mg wet tissue
6
5
4
3
2
1
0
**
**
Naive 24h
Naive 7days
Experienced 24h
Experienced 7days

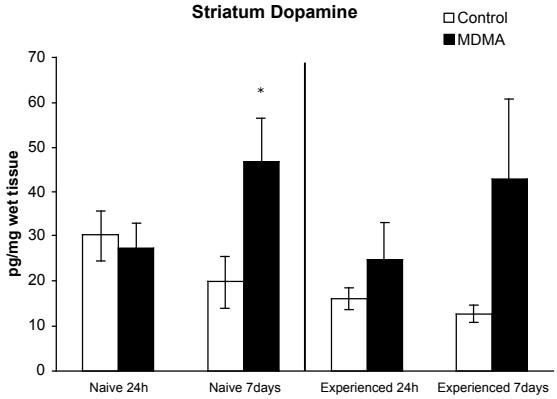
C


NAcc Dopamine
Control
MDMA
pg/mg wet tissue
50
45
40
35
30
25
20
15
10
5
0
*
**
Naive 24h
Naive 7days
Experienced 24h
Experienced 7days

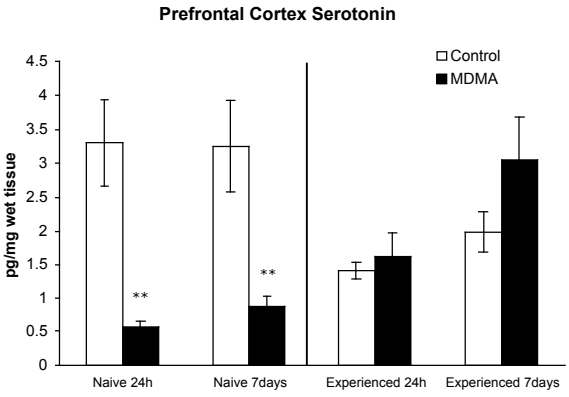
D


Striatum Serotonin
Control
MDMA
pg/mg wet tissue
3.5
3
2.5
2
1.5
1
0.5
0
*
**
Naive 24h
Naive 7days
Experienced 24h
Experienced 7days

E


Striatum Dopamine
Control
MDMA
pg/mg wet tissue
70
60
50
40
30
20
10
0
*
Naive 24h
Naive 7days
Experienced 24h
Experienced 7days

F


Prefrontal Cortex Serotonin
Control
MDMA
pg/mg wet tissue
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
**
**
Naive 24h
Naive 7days
Experienced 24h
Experienced 7days

G

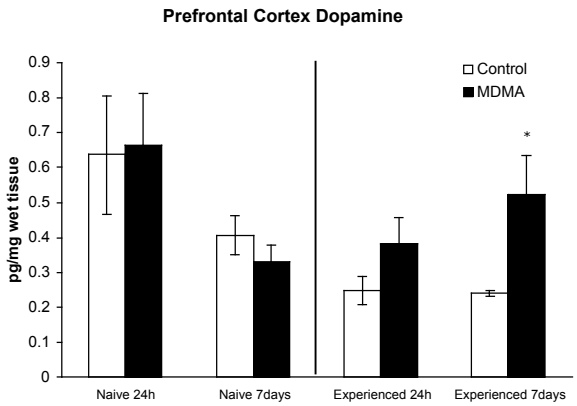

Prefrontal Cortex Dopamine
pg/mg wet tissue
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control
MDMA
*
Naive 24h
Naive 7days
Experienced 24h
Experienced 7days

Table 1.3 Regional brain concentrations of DA, DOPAC, HVA, 5-HT, and 5-HIAA (expressed as pg/mg wet tissue) for all the groups tested.

HIPPOCAMPUS

| | NAIVE | | | | EXPERIENCED | | | |
|---|---|---|---|---|---|---|---|---|
| | 24 HOURS | | 7 DAYS | | 24 HOURS | | 7 DAYS | |
| | CONTROL | MDMA | CONTROL | MDMA | CONTROL | MDMA | CONTROL | MDMA |
| 5-HT | 0.61±0.11 | 0.35±0.07* | 0.91±0.09 | 0.58±0.09* | 0.61±0.06 | 0.32±0.04** | 0.73±0.06 | 0.55±0.09 |
| 5-HIAA | 1.58±0.31 | 2.46±0.08** | 2.62±0.28 | 3.01±0.43 | 2.65±0.08 | 1.59±0.25** | 2.64±0.16 | 1.26±0.14** |

NUCLEUS ACCUMBENS

| | NAIVE | | | | EXPERIENCED | | | |
|---|---|---|---|---|---|---|---|---|
| | 24 HOURS | | 7 DAYS | | 24 HOURS | | 7 DAYS | |
| | CONTROL | MDMA | CONTROL | MDMA | CONTROL | MDMA | CONTROL | MDMA |
| DA | 14.48±2.19 | 18.61±2.39 | 13.45±3.74 | 36.19±9.42* | 15.56±3.04 | 14.95±4.52 | 9.72±0.93 | 21.65±3.11** |
| DOPAC | 9.71±3.84 | 14.33±2.02 | 14.93±1.13 | 13.87±1.02 | 11.95±2.58 | 17.49±4.32 | 17.06±1.26 | 18.96±5.80 |
| HVA | 2.10±0.67 | 3.37±0.54 | 4.7±0.2 | 3.48±0.48* | 4.44±0.8 | 2.99±0.4 | 3.62±0.34 | 3.46±0.67 |
| 5-HT | 2.39±0.46 | 0.92±0.13** | 1.97±0.35 | 4.06±1.07 | 2.09±0.25 | 1.31±0.67 | 1.77±0.22 | 0.99±0.14** |
| 5-HIAA | 2.39±0.77 | 2.76±0.21 | 3.89±0.24 | 2.23±0.15** | 3.42±0.33 | 2.35±0.14** | 4.10±0.44 | 2.33±0.39* |

STRIATUM

| | NAIVE | | | | EXPERIENCED | | | |
|---|---|---|---|---|---|---|---|---|
| | 24 HOURS | | 7 DAYS | | 24 HOURS | | 7 DAYS | |
| | CONTROL | MDMA | CONTROL | MDMA | CONTROL | MDMA | CONTROL | MDMA |
| DA | 30.01±5.61 | 27.19±5.61 | 19.72±1.41 | 46.91±9.34* | 16.01±2.46 | 24.59±8.56 | 12.60±1.96 | 42.91±17.9 |
| DOPAC | 8.44±3.70 | 14.06±2.5 | 12.52±5.84 | 16.96±2.95 | 9.64±1.76 | 11.40±3.61 | 15.79±1.82 | 12.75±3.22 |
| HVA | 2.63±0.79 | 3.94±0.65 | 4.11±0.70 | 4.72±0.4 | 3.85±0.48 | 2.86±0.65 | 4.24±0.56 | 3.95±1.17 |
| 5-HT | 1.98±0.27 | 1.38±0.59 | 1.36±0.14 | 2.97±0.25* | 1.50±0.04 | 1.01±0.12** | 1.02±0.11 | 2.12±0.66 |
| 5-HIAA | 1.91±0.3 | 2.26±0.21 | 3.54±0.36 | 2.31±0.36 | 3.3±0.17 | 2.06±0.27** | 2.75±0.27 | 2.47±0.38 |

(con't)

PREFRONTAL CORTEX

| | NAIVE | | | | EXPERIENCED | | | |
|---|---|---|---|---|---|---|---|---|
| | 24 HOURS | | 7 DAYS | | 24 HOURS | | 7 DAYS | |
| | CONTROL | MDMA | CONTROL | MDMA | CONTROL | MDMA | CONTROL | MDMA |
| DA | 0.63±0.16 | 0.6±0.15 | 0.4±0.05 | 0.32±0.04 | 0.24±0.03 | 0.38±0.07 | 0.24±0.01 | 0.52±0.11* |
| DOPAC | 0.2±0.03 | 0.27±0.02 | 0.27±0.03 | 0.38±0.06 | 0.26±0.02 | 0.23±0.01 | 0.26±0.01 | 0.24±0.01 |
| HVA | 0.09±0.05 | 0.08±0.01 | 0.14±0.01 | 0.19±0.01 | 0.22±0.03 | 0.04±0.01** | 0.22±0.01 | 0.05±0.01** |
| 5-HT | 3.3±0.63 | 0.56±0.08** | 3.24±0.67 | 0.88±0.15** | 1.41±0.11 | 1.61±0.37 | 1.98±0.3 | 3.05±0.62 |
| 5-HIAA | 2.19±0.48 | 2.99±0.41 | 3.46±0.37 | 3.25±0.33 | 3.80±0.35 | 2.36±0.33** | 3.08±0.35 | 2.28±0.3 |

## DISCUSSION

Ecstasy (or MDMA) popularity spreads worldwide among teenagers, but little is known regarding the long-term effects of repetitive MDMA exposure. Although most studies focus on the rewarding effects of other psychostimulants, MDMA has been proved to maintain high rates of self-administration in rats (Daniela et al., 2004; Schenk et al., 2003). In agreement with those studies, we have shown that (+/-)-MDMA acts as a reinforcer in drug-naïve animals (Fig. 2.3) when acquisition conditions are used (10 days pre-exposure using 1.0 mg/kg (+/-)-MDMA per infusion, see experimental design).

MDMA is a unique compound in the sense that it causes a massive release of 5-HT in several brain regions acting like selective 5-HT releasing agents (i.e., fluoxetine), but also promotes the release of DA like selective releasers (i.e., amphetamine). In agreement with previous studies (Kankaanpaa et al., 1998; O'Shea et al., 2005), we found (+/-)-MDMA (3mg/kg) elevates extracellular DA and 5-HT (see Fig. 6.3 A and B, respectively) in the NAcc. However, contrarily to what has been previously reported MDMA was more potent 5-HT than DA releaser in both naïve and drug-experienced animals (approx. 800 versus 250% increase). One possible explanation for this disparity might be in the present study MDMA was intravenously self-administered, whereas non-voluntary subcutaneous, or intraperitoneal routes were used in other studies. Previous studies have suggested enhanced 5-HT transmission correlates negatively with increase self-administration of drugs of abuse (Howell and Byrd, 1995; Smith et al., 1986; Wee et al., 2005; Yu et al., 1986). Yet, increased 5-HT activity has been both associated with potentiation or inhibition of MDMA-induced reward (Fletcher et al., 2002; Harrison and

Markou, 2001). Our results show MDMA self-administration elicited the same degree of 5-HT release in naïve and experienced rats in the NAcc (see Fig 6.3 B).

There has been also overwhelming evidence linking increased DA neurotransmission with drugs of abuse reinforcement (Anderson and Pierce, 2005; Di Chiara, 1995; Wee et al., 2005; Wise, 1978). However, our data indicate MDMA effect on NAcc DA levels was more pronounced in naïve animals during the first 10 min after lever activation (see Fig. 6.3A). It is important to mention, no differences in response latency (time that elapses between the lever activation and the lever response) were observed between naïve and experienced MDMA groups (data not shown). Multiple lines of evidence suggest long-term administration of drugs of abuse lead to neuronal plasticity within the DA system and 5-HT systems (Bruijnzeel and Markou, 2005; Staley and Mash, 1996; Tupala et al., 2003). For example, repetitive exposure to cocaine can decrease DA release responses in the NAcc (Hurd et al., 1989). Under these circumstances, self-administration experiments have shown animals will increase the number of lever presses in other to achieve the same neurochemical response. This suggests the increase in the number of lever responses observed with MDMA experienced (see Fig. 2.3) could be the result of a relative attenuation in NAcc DA release (see Fig. 6.3A) in experienced animals.

Many studies have reported the locomotor activating effects of large doses of MDMA when injected to rodents (Callaway et al., 1990; Fantegrossi et al., 2003; Gold and Koob, 1989; Spanos and Yamamoto, 1989). However, at low and moderate doses (2.5-5.0 mg/kg), MDMA fails to induce hyperlocomotion compared to basal activity levels (Matthews et al., 1989; McNamara et al., 1995; Spanos and Yamamoto, 1989). To

date, no study has investigated the effect of daily long-term MDMA self-administration on rat locomotor activity. Figure 4.3 shows MDMA self-administration did not elicit hyperactivity on days 1-8 compared to baseline levels (daily average intake 4mg/kg), however, as MDMA exposure increases an enhancement in locomotion was observed and MDMA significantly induced hyperactivity by day 13. The present study also confirms MDMA induces behavioral sensitization (Dafters, 1995; McCreary et al., 1999; Spanos and Yamamoto, 1989). Considering daily MDMA intake was approximately the same throughout the self-administration period, no increase in locomotor activity was observed in drug-naïve animals after MDMA self-administration while a progressive increase in locomotor counts was evident with drug experience (see Fig. 4.3). While the mechanisms of MDMA-induced behavioral sensitization is not fully understood, stimulation of 5-$HT_{2C}$ receptors is known to block MDMA-associated locomotor sensitization in rats previously exposed to MDMA (Ramos et al., 2005). In addition, activation of 5-$HT_{2C}$ is known to reduce MDMA-induced locomotor activity in naïve animals (Bankson and Cunningham, 2002). Thus, MDMA-induced behavioral sensitization observed with MDMA experience might be the result of post-synaptic down-regulation of 5-$HT_{2C}$ receptors. In fact, studies have shown chronic treatment with 5-HT uptake inhibitors results in down-regulation of many 5-$HT_2$ receptors (Gray and Roth, 2001; Klimek et al., 1994), including 5-$HT_{2C}$ receptors (Bristow et al., 2000; Millan, 2005).

The present study also demonstrated MDMA-induced acute specific behaviors of the 5-HT syndrome are altered by chronic MDMA self-administration (see Fig. 5.3). Tolerance developed to the low body posture component while MDMA-induced snout secretions increased with continued self-administration of MDMA. Long-term and

continuous administration of drugs of abuse is associated with the development of tolerance, and evidence indicates molecular adaptations that occur with repetitive drug exposure might be responsible for these phenomena (Hammer et al., 1997). Likewise, chronic administration of antidepressants induces neuroadaptive changes involving up and down-regulation of specific DA and 5-HT receptors (Matrisciano et al., 2005; Matrisciano et al., 2002; Moret and Briley, 1990).

While a majority of studies have reported MDMA-mediated increase in body temperature, MDMA has been shown to have no effect on body temperature, or induce hyperthermia or hypothermia depending on the experimental conditions (e.g. ambient temperature, restraint; (Dafters, 1995; Miller and O'Callaghan, 1995). In addition, some authors have reported MDMA-induced hypothermia in rats kept at room temperatures (Bexis and Docherty, 2006; Malberg and Seiden, 1998; Malpass et al., 1999). Our results indicate moderate amounts of self-administered MDMA initially induce a reduction in rectal temperature, however tolerance occurs by day 8, and MDMA self-administration did not influence core temperature thereafter (see Fig. 3.3). D-fenfluramine (a potent 5-HT releaser) induces hypothermia under normal and low ambient temperatures and 5-$HT_{1A}$, 5-$HT_{2B}$, and $D_2$ receptor activation might contribute to this effect (Cryan et al., 2000; Malberg and Seiden, 1997). Since MDMA elicited the same degree of NAcc 5-HT efflux in naïve and MDMA-experienced animals (see, Fig. 6.3B), we hypothesize the pronounced increase in 5-HT release observed in naïve animals might be responsible for the initial decrease in rectal temperature (see Fig. 3.3), but receptor subsensitization following chronic exposure might reflect the subsequent lack of hypothermia.

Finally, our results indicate long-term voluntary MDMA administration in rats induced short and long-term changes in tissue monoamines and metabolites in various brain regions (see Fig. 8.3). Although levels of 5-HT were decreased 24 hours after treatment, hippocampal 5-HT levels recovered 7 days after microdialysis in (+/-)-MDMA experienced animals. In addition, while 5-HT turnover (5-HIAA/5-HT ratio) was increased in the hippocampus of naïve animals 24 hours after an acute 3mg/kg MDMA infusion, levels of 5-HT and 5-HIAA were both reduced at the same time point in (+/-)-MDMA experienced animal. Seven days after treatment, NAcc and PFC DA levels were elevated in experienced animals compared to control animals. Increased tissue DA levels in these regions might occur via stimulation of presynaptic DA $D_2$ (or $D_3$) autoreceptors that results in inhibition of DA release in those same regions (Cass and Gerhardt, 1994; Gobert et al., 1995; Whetzel et al., 1997). This hypothesis is consistent with our results showing decreased NAcc DA release in experienced animals.

In conclusion, the present study found chronic (+/-)-MDMA self-administration results in physiological, neurochemical and behavioral changes that likely reflect synaptic plasticity. Like with other drugs of abuse, long-term MDMA-induced alterations within the mesocorticolimbic pathway might contribute to MDMA addicted behavior. In addition, our results demonstrate long-term (+/-)-MDMA self-administration induces behavioral sensitization in rats, a phenomenon that is thought to contribute to drug craving and relapse.

Table 2.3 Average tissue levels of monoamines and metabolites expressed as μg/g Comparison with literature. Our results are included inside braces.

**STRIATUM**

| REFERENCE | DA | DOPAC | HVA | 5-HT | 5-HIAA |
|---|---|---|---|---|---|
| 1 | | | | 0.441 | 0.570 |
| 2 | | | | | |
| 3 | | | | 0.441 | 0.570 |
| 4 | | | | 0.35 | 0.42 |
| 5 | 8.50-10.45 | 0.81-1.09 | 0.62-0.79 | 0.533 | 0.557 |
| 6 | 8.2 | 1.19 | 0.582 | | |
| 7 | 8.7 | | | 0.23 | |
| 8 | | | | | |
| 9 | 9.05-10.47 | 1.67-4.2 | | | |
| 10 | 7.54 | | | 0.522 | 0.350 |
| 11 | ~8-10 | ~0.80 | ~0.45 | ~0.40 | ~0.40 |
| 12 | 6.09 | 3.21 | 0.78 | 0.363 | 0.492 |
| **13 *** | 16.26 | 2.10 | 1.06 | 0.89 | 1.02 |
| 14 | | | | | |
| Sham | 6.25 | 2.87 | 0.972 | 0.367 | 0.442 |
| Saline dialysis-24h | 5.69 | 0.33 | 0.261 | 0.422 | 0.407 |
| Saline dialysis-7days | 3.74 | 2.10 | 0.75 | 0.289 | 0.754 |

***** Discrepancies with all other references

**FRONTAL CORTEX**

| REFERENCE | DA | DOPAC | HVA | 5-HT | 5-HIAA |
|---|---|---|---|---|---|
| 1 | | | | 0.515 | 0.28 |
| 2 | | | | 0.420 | 0.361 |
| 3 | | | | 0.515 | 0.287 |
| 4 | | | | 0.518 | 0.218 |
| 5 | | | | | |
| 6 | | | | | |
| 7 | Undetected | | | | |
| 8 | | | | 0.2 | 0.03 |
| 9 | 0.078 PFC | 0.035 PFC | | | |
| 10 | 0.285 PFC | | | 0.521 PFC | 0.172 PFC |
| 11 | ~0.07 PFC | ~0.03 PFC | ~0.02 PFC | ~0.8 PFC | ~0.4 PFC |
| 12 | | | | | |
| 13 | 0.057 PFC | 0.015 PFC | 0.026 PFC | 0.464 PFC | 0.186 PFC |
| 14 | | | | | |
| 15 | | | | | |
| Sham | 0.099 | 0.100 | 0.045 | 0.312 | 0.517 |
| Saline dialysis-24h | 0.12 | 0.013 | 0.091 | 0.954 | 0.449 |
| Saline dialysis-7days | 0.048 | 0.055 | 0.038 | 0.419 | 0.700 |

(con't)

**HIPPOCAMPUS**

| REFERENCE | DA | DOPAC | HVA | 5-HT | 5-HIAA |
|---|---|---|---|---|---|
| 1 | | | | 0.359 | 0.412 |
| 2 | | | | | |
| 3 | | | | 0.359 | 0.412 |
| 4 | | | | 0.359 | 0.338 |
| 5 | | | | | |
| 6 | | | | | |
| 7 | Undetected | | | 0.23 | |
| 8 | | | | 0.15 | 0.07 |
| 9 | | | | | |
| 10 | 0.0789 | | | 0.592 | 0.407 |
| 11 | | | | | |
| 12 | 0.031 | 0.039 | 0.006 | 0.410 | 0.684 |
| 13 | | | | | |
| 14 | | | | | |
| 15 | | | | | |
| Sham | Undetected | Undetected | Undetected | 0.134 | 0.398 |
| Saline dialysis-24h | Undetected | Undetected | Undetected | 0.107 | 0.219 |
| Saline dialysis-7days | Undetected | Undetected | Undetected | 0.193 | 0.500 |

**Nucleus Accumbens**

| REFERENCE | DA | DOPAC | HVA | 5-HT | 5-HIAA |
|---|---|---|---|---|---|
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |
| 5 | | | | | |
| 6 | | | | | |
| 7 | | | | | |
| 8 | | | | | |
| 9 | | | | | |
| 10 | | | | | |
| 11 | ~10 | ~1.1 | ~0.5 | ~0.7 | ~0.4 |
| 12 | | | | | |
| 13 | 10.19 | 1.66 | 0.77 | 1.56 | 0.97 |
| 14 | | | | | |
| 15 | | | | | |
| Sham | 5.36 | 2.92 | 0.631 | 0.678 | 0.575 |
| Saline dialysis-24h | 2.80 | 1.01 | 0.277 | 0.488 | 0.323 |
| Saline dialysis-7days | 2.55 | 2.51 | 0.857 | 0.420 | 0.827 |

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Basal extracellular DA and 5-HT levels in the Nacc --- Comparison with the literature: Our values are from basal naïve saline animals

| Reference | 5-HT pg/μl | DA pg/μl | 5-HT pmol/10μl | DA pmol/10μl | 5-HT nM | DA nM |
|---|---|---|---|---|---|---|
| 1 | | | | 0.0126 | | |
| 2 | | | | | 0.66 | 1.54 |
| 3 | | 0.25 | | | | |
| 4* | | | | | 1.2 | 2.6 |
| 5 | | 0.6-0.8 | | | | |
| 6 | | | | | 4.58 | 14.14 |
| 7 | | | | | | |
| Our values | 0.196 | 0.401 | 0.010 | 0.021 | 0.921 | 2.117 |
| | | | | | | |

*Same coordinates I used

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## CHAPER 3 SUMMARY

There is a general consensus on the importance of the mesolimbic dopamine pathway in the actions of most drugs of abuse. Hence, tolerance to the behavioral effects of psychostimulants has been correlated with adaptive changes in the DA system. Yet, as previously discussed, MDMA pharmacology is complex in the sense many neurotransmitters are involved.

Our results show repetitive self-administration of MDMA for an extended period induces tolerance to MDMA-mediated hypothermia and low body posture serotonin syndrome behavior. The concurrence of progressive normalization in body temperature and gradual rise in locomotor activity with MDMA exposure suggested both effects might be linked to the other. In fact, changes in activity during the circadian rhythm synchronize with core temperature rhythms (Benstaali et al., 2001; Decoursey et al., 1998; Weinert and Waterhouse, 1998). In order to test this hypothesis, it would be of interest to measure non-shivering thermogenesis (the most important source of heat in small mammals) and locomotor activity during daily MDMA and saline self-administration sessions. Daily variations in metabolic rates, tail blood pressure, cutaneous blood flow, core and tail skin temperature readings, and locomotor counts would give an idea of the importance of MDMA-mediated increase in activity as a source of heat. In addition, the acute decrease in rectal temperature observed from days 1-8 might be a consequence of MDMA-associated low body posture that is accompanied by full body stretch and splayed hind limbs. For this reason, thermal conductance of the animal (a measure of heat transfer from the body to the environment) and daily tail temperature measurements would determine the importance of this behavior in shaping daily variations in temperature. Alternatively, subsensitivity of specific central or peripheral

DA, 5-HT, or NA receptors might underlie the mechanisms of MDMA tolerance. It would be important, in future studies, to test this hypothesis using specific receptor agonist or antagonist, even by the use of microinjections into target regions to determine the involvement of specific brain areas in MDMA-mediated disruption of body temperature and tolerance with MDMA experience.

In general, acute and long-term administration of large doses of MDMA induces long-term 5-HT and 5-HIAA depletions. In this study, chronic daily MDMA self-administration caused a decrease in 5-HT levels in the hippocampus and NAcc. However, this response was reversed in the hippocampus (an area densely populated by 5-HT nerve terminals) after 7 days of MDMA abstinence, while a marked elevation in NAcc DA was detected at the same time point. The results from this study suggest changes in the content of neurotransmitters in various brain regions correlate with the development of tolerance or behavioral sensitization to MDMA, and are the result of neuroadaptational changes that occur with increased MDMA exposure. Interestingly, levels of prefrontal 5-HT were drastically reduced in naïve animals at both 24 hours and 7 days after a 3 mg/kg MDMA infusion, while levels of 5-HIAA remained unchanged. It would be of particular value to extend this study by measuring PFC 5-HT release using *in vivo* microdialysis in naïve and experienced MDMA rats.

Several explanations might be proposed to account for the lack of neurotoxicity after long-term MDMA self-administration. For example, hyperthermia plays an important role in MDMA-mediated toxic effects. In the present study animals did not develop hyperthermic responses and this might explain why levels of hippocampal 5-HT were not reduced 7 days after chronic MDMA use. In this were the case these data would

confirm the important contribution of elevated core temperature in MDMA-associated damage. One way to test this hypothesis would be to study long-term MDMA self-administration when animals are maintained at high ambient temperatures. Another possibility could in this study we used voluntary moderate doses, as opposed to large experimenter-delivered amounts of MDMA.

The ability of moderate MDMA intake to disrupt fundamental homeostatic mechanisms of thermoregulation indicates MDMA's alarming potential for biological disorder. There is no doubt MDMA users experience many side effects (e.g. increase in blood pressure, hyperthermia, hallucinations) many of which become life threatening especially with heavy consumption. While most of these adverse effects appear to be short-lived, chronic MDMA intake in humans might have long-lasting sequelae. Further studies investigating physiological changes induced by self-administered MDMA will help to determine relevant risk factors associated with recreational MDMA use.

# 5-$HT_{2C}$ RECEPTOR ANTAGONISM ENHANCES MDMA-INDUCED DA RELEASE AND LOCOMOTOR BEHAVIOR


## ABSTRACT

3, 4-methylenedioxymethamphetamine (MDMA) is a psychostimulant which mechanism of action has not yet been determined. MDMA actions on serotonin (5-HT) and dopamine (DA) systems underlie the behavioral and neurochemical responses induced by this drug. In this study, we evaluated the specific role of 5-$HT_{2C}$ receptors in MDMA-induced nucleus accumbens (NAcc) DA and 5-HT release using *in vivo* microdialysis. Rats were pre-treated with either saline, or SB242084 (1mg/kg), prior to a single self-administered infusion of MDMA (3mg/kg), or saline. MDMA elicited a more pronounced increase in extracellular 5-HT (aprox. 1000x) than in DA (aprox. 200x) in saline-pretreated rats. Pre-treatment with SB242084 induced a significant greater increase in MDMA-mediated DA release compared to saline pretreated rats, while no change in 5-HT release was observed. Self-administration of MDMA caused an increase in locomotor activity in SB242084 pretreated rats, but had no effect in saline-pretreated animals. We also evaluated MDMA-induced low body posture (LBP) specific behavior and found LBP-no net movement characteristic of saline pretreated animals, while LBP was accompanied by movement in SB242084 pretreated rats. In conclusion, these results suggest 5-$HT_{2C}$ receptors inhibitory role in MDMA-associated NAcc DA release, and locomotor activation.

## INTRODUCTION

Serotonin (5-HT) produces its effects upon interaction with specific 5-HT receptors. To date, seven different families of membrane-bound receptors have been cloned and identified (5-$HT_1$ though 5-$HT_7$; (Hoyer et al., 1994; Peroutka, 1988; Saudou and Hen, 1994). With the exemption of 5-$HT_3$ receptors, which are ligand-gated ion channels (Derkach et al., 1989), all other 5-HT receptors belong to the G-protein-coupled receptors (GPCR) superfamily and are widely expressed in the central and peripheral nervous system (Pazos et al., 1985; Pazos and Palacios, 1985). The 5-$HT_2$ superfamily are seven-transmembrane receptors and consists of the A, B and C subtypes (Humphrey et al., 1993) that are positively coupled to Gq proteins and mediate the activation of phospholipase C and phospholipase $A_2$ (Boess and Martin, 1994; Sanders-Bush, 1990). The 5-$HT_{2C}$ receptors were initially characterized in porcine choroids plexus (Pazos et al., 1984), and further identified in various regions including the substantia nigra, nucleus accumbens (NAcc), hippocampus, cerebral cortex and olfactory tubercles (Palacios, 1991; Rick et al., 1995).

3,4-Methylenedioxymethamphetamine (MDMA) is a popular amphetamine derivative that exerts its psychostimulant effects in part through the massive release and subsequent interaction of 5-HT with its receptors (Battaglia and De Souza, 1989; Gough et al., 1991; Gudelsky and Nash, 1996; Nichols et al., 1982). In addition, MDMA induces the release of dopamine (DA) from nerve terminals that results in enhanced DA neurotransmission in various brain regions (Colado et al., 1999; Gough et al., 1991; Gudelsky et al., 1994; Yamamoto and Spanos, 1988). In particular, MDMA exposure activates mesolimbic DA neurons in the ventral tegmental area (VTA) that project to the NAcc (Cadoni et al., 2005; Gold et al., 1989; Yamamoto and Spanos, 1988). It is widely

accepted activation of these brain regions mediates the behavioral and rewarding effects of psychostimulants, such as MDMA (Di Chiara et al., 2004; Gold et al., 1989; Kelly and Iversen, 1976; Schlaepfer et al., 1997). Several studies suggest DA transmission can be modulated via 5-HT binding to specific 5-HT receptors (Bankson and Yamamoto, 2004; Ichikawa and Meltzer, 1995; Schmidt et al., 1992; Yamamoto et al., 1995). In particular, mesolimbic DA cell firing can be indirectly inhibited through 5-$HT_{2C}$ activation and subsequent increase GABA transmission (Bankson and Yamamoto, 2004).

Systemic administration of high to moderate acute doses of MDMA elicits hyperactivity in rodents (Daniela et al., 2004; Gold et al., 1988; Kehne et al., 1996), while chronic exposure causes behavioral sensitization (McCreary et al., 1999; Modi et al., 2006; Ramos et al., 2004). In addition, MDMA exposure induces many of the behavioral features characteristics of the "serotonin syndrome" (e.g. low body posture, piloerection, forepaw treading; Colado et al., 1993; Spanos and Yamamoto, 1989). It is widely accepted MDMA administration exerts a massive release of DA and 5-HT from presynaptic termianals (Gudelsky and Nash, 1996; Nash and Brodkin, 1991; Yamamoto and Spanos, 1988) and both neurotransmitters have been implicated in MDMA-induced unique behavior (Bankson and Cunningham, 2001; Bubar et al., 2004; Fletcher et al., 2002; McCreary et al., 1999; Ramos et al., 2005; Ross et al., 2006). The use of MDMA causes an increase in body temperature in humans (Freedman et al., 2005; Henry et al., 1992), and studies carried out with laboratory animals corroborate MDMA induces hyperthermia (Colado et al., 2001; Nash et al., 1988; O'Loinsigh et al., 2001; O'Shea et al., 1998) and affects general mechanisms of thermoregulation (Malberg and Seiden, 1998). Evidence strongly supports DA and 5-HT receptor activation also play a key role in this effect (Mazzola-Pomietto et al., 1997; Mechan et al., 2002), although MDMA is

also known to act as an agonist at 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors (Nash et al., 1994). The vast majority of the literature suggests MDMA effect on core temperature is influenced in part by small changes in ambient temperature (Dafters, 1994; Gordon et al., 1991; Malberg and Seiden, 1998).

The two-state model of GPCR function defines inverse antagonist as (Strange, 2002) a compound that switches the receptor to an inactive state in the absence of the endogenous ligand (e.g. 5-HT), and by doing so, they can reduce basal activity. SB242084 (6-chloro-5-methyl-1-(6-(2-methylpiridin-3-yloxy)pyridin-3-yl carbamoyl) indoline ) is a selective 5-$HT_{2C}$ inverse agonist. Evidence suggests SB242084 blockage of 5-$HT_{2C}$ receptors mediates an increase in DA transmission in various brain regions, including the NAcc (Gobert et al., 2000; Millan et al., 1998). In this study, we investigated the specific role of 5-$HT_{2C}$ receptors in MDMA-induced behavior and possible modulation of DA and 5-HT release in the NAcc. To resemble a model of recreational MDMA consumption, animals self-administered 3mg/kg MDMA, and physiological, neurochemical and behavioral changes were measured.

## MATERIALS AND METHODS

### Animals and Surgeries

Male Sprague Dawley rats (5 week old) were purchase from Charles River (Kingston, NY), and were house in groups of 3, provided with tap water and food *ad libitum*, in a room maintained at 20 ± 1°C, with a reverse light cycle (lights on from 8:00p.m. – 8:00 a.m.). Rats were handled daily for 2 weeks prior the beginning of the study to minimize stress. When animals reached an average body weight of 200g they

received lever-press training for sucrose pellets on a fixed ratio 1 (FR1) schedule for a minimum period of 7 days. Subsequently, rats were surgically implanted with a jugular catheter and stereotaxic surgeries were performed as previously described (Ikegami and Duvauchelle, 2004). Microdialysis probes aimed the nucleus accumbens (flat brain coordenates: AP 2.0mm, DV 2.5mm, ML 1.2mm. Rats were singly housed after surgery and allowed to recover for 7 days. All experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Drugs**

(+/-) 3,4-methylenedioxymethamphetamine HCl (MDMA) was obtained through NIDA Drug Inventory Supply and Control. MDMA was dissolved in a sterile 0.9% sodium chloride solution (saline) and concentration for individuals adjusted for body weight to make a test concentration of 3mg/kg delivered in a volume of 0.1ml. SB242084 was purchased from Sigma and dissolved in saline containing 8% 2-hydroxypropyl-ß-cyclodextrin and 25 mM citric acid, heated with continuous stirring and allowed to reach room temperature. SB242084 was intravenously administered through the catheter at a dose of 1mg/kg in a volume of 0.2ml.

**Dialysis probes and *in vitro* probe recovery analysis**

Microdialysis probes were constructed using polyethylene 20 tubing as the inlet and fused silica tubing (75 $\mu$m i.d.) inserted into a 2.5mm section of cellulose (13 kDalton cut-off; Spectrum, Houston) fitted through a 26 gauge internal cannula (Plastic One, Roanoke, VA). The outlet consisted of fused silica coated with polyethylene 50

tubing. To determine recovery rates for each probe, Hamilton syringes were filled with freshly prepared filtered artificial cerebral spinal fluid (ACSF) solution, and pumped continuously through the probe at a rate of 1.63 $\mu$l/min. Probes were placed in a beaker containing ascorbate (1%), 4nM DA, and 4nM 5-HT maintained at 37°C. 10-min dialysis samples from each probe were collected, and assayed by high performance liquid chromatography (HPLC) coupled to ampirometric detection (Shizeido Capcell, 1.5 mm X 50 mm, 3 $\mu$m particle size column, ESA 5041 ampirometric cell detector with the oxidizing potential set to +200 mV, sensitivity 100 pA, guard cell potential 400 mV; ESA, Inc., Chelmsford, MA). The mobile phase contained 150 mM Na2H2PO4, 50 $\mu$M EDTA disodium salt, 4.76 mM citric acid, 4.5–6.0 mM sodium dodecyl sulfate, 12.5% (v/v) acetonitrile, 12.5% (v/v) methanol, pH 5.6, constant flow rate of 0.2 $\mu$l/min. Probe recovery was calculated by comparing the peak heights of each dialysate and those from a 25% recovery standard solution. Data was collected and analyzed using an ESA Model 500 Data station. Average DA and 5-HT recoveries were ~ 13-15%.

**Experimental procedure**

Microdialysis test sessions were conducted in operant chambers (28X22X21 cm, Med Associates, St. Albans, VT). A single retractable operant lever was located on the right wall. Inside the chamber, a swivel was attached at one end by Tygon tubing to a syringe mounted on a motor-driven syringe pump (Razel, St. Albans, VT)) located outside the chamber. At the other end of the swivel a cannula connector with spring (Plastic One, Roanoke, VA) was attached to the animal's catheter. A house light was located at the top of the left metal wall. At the beginning of the experiment 10-min basal samples were collected. Subsequently, animals were infused with either SB242084 or saline (Test 1), and another set of three 10-min samples was collected. At this point the

lever was inserted into the chamber and animals self-administered either MDMA or saline (Test 2). Six consecutive dialysis samples were collected at 10-min intervals during the Test 2 period. Locomotor activity counts were recorded during baseline, Test 1 and 30-min of Test 2 session. Dialysate DA and 5-HT content were assessed using HPLC coupled to amperimetric detection (as described above). The limit of detection (at S/N ratio 3) was 0.36 fg/sample for DA, and 0.38 fg/sample for 5-HT.

In addition, animals were videotaped 30 minutes after pre-treatment and self-administration during 1-minute recordings. Head weaving (lateral side-to-side movements of the head with no net locomotion, while the animals abdomen in contact with the floor) and low body posture-movement (the animal's abdomen is in contact with the floor and is accompanied by movement of forelimbs and hindlimbs) were rated according to the following scale: 0=absent; 1=occasional (the behavior was present for less than 20 seconds, during the one-minute observation period); 2=frequent (the behavior was present for less than 30 seconds); and 3=constant (the behavior was present for more than 30 seconds). An observer, blinded to the treatment given to the animals, performed ratings.

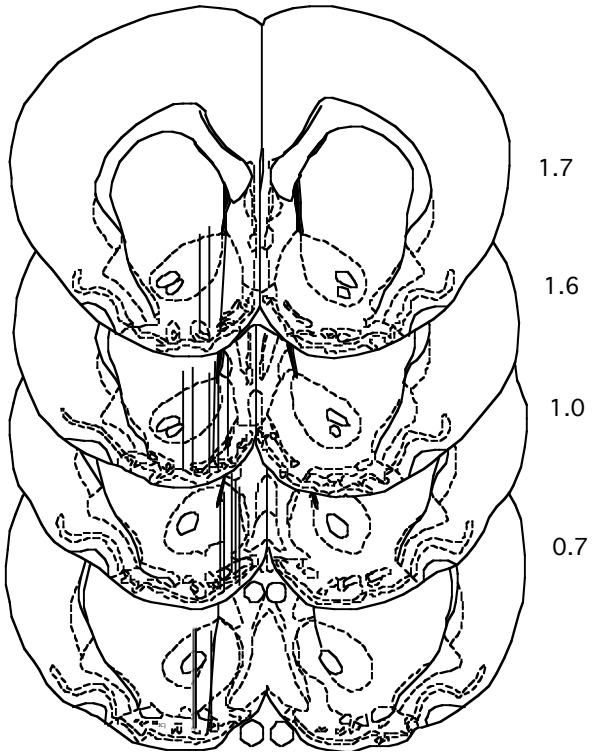
**Rectal temperatures**

Rectal temperatures of each animal were monitored before and after the microdialysis test session using a V911 digital thermometer (Procter & Gamble, Cincinnati, OH), probe inserted to a depth of approximately 4 cm.

**Histology**

After completion of the experiment, animals were euthanized, and microdialysis probes placement into the NAcc confirmed with histological analyses of 60 $\mu$m coronal sections stained with cresyl violet. Figure 1.4 shows the schematic representation of the active dialysis probe membrane region in the NAcc for all animals.

Figure 1.4 Schematic representation of the active dialysis probe membrane region in the NAcc of all treated animals (n=18) that completed the dialysis experiment. Numbers depicted next to each brain slice indicate the mm anterior to bregma. The diagram was drawn with the assistance of the atlas of Paxinos and Watson (Paxinos and Watson, 1997).



### Statistical Analyses

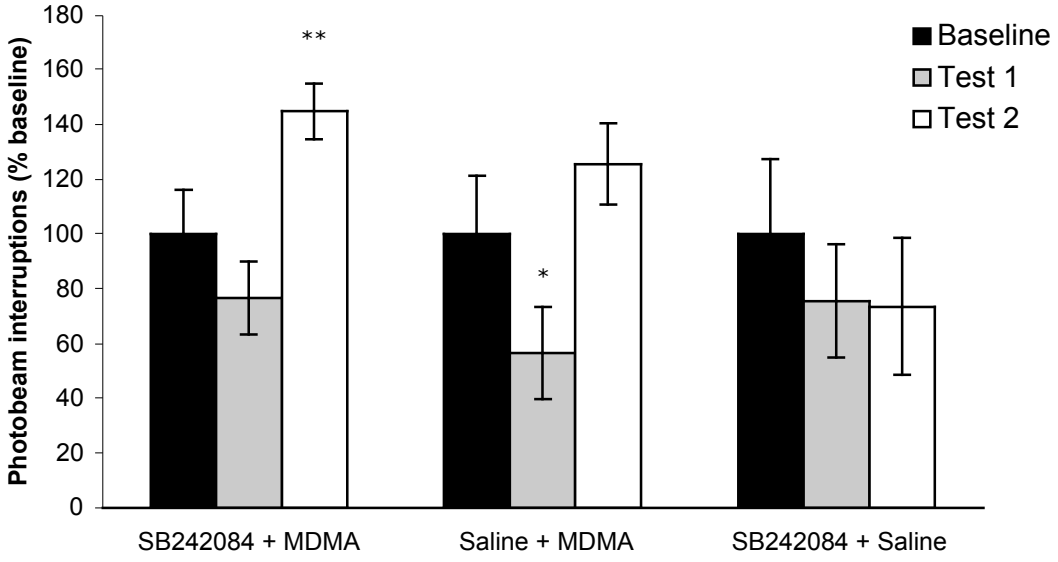
Data were analyzed using two-way repeated measures ANOVA. When a significant effect was detected, post-hoc comparisons (Fisher LSD) of each group to its respective control were performed to determine specific differences.

## RESULTS

### Effect of drug treatment on locomotor activity

A two-way repeated measures ANOVA showed a significant group effect ($F(2,15)=4.53$; $p=0.0288$), time effect ($F(2,30)=8.37$; $p=0.0013$), and group X time interaction ($F(4,30)=3.54$; $p=0.0176$). Posthoc test indicate SB242084 pre-treatment resulted in a significant increase in total locomotor counts in animals that self-administered MDMA compared to baseline (see Fig. 2.4; $p<0.01$). However, when animals where pre-treated with saline no significant increase in locomotor activity was recorded after MDMA self-administration compared to basal levels. SB242084 in combination with saline did not induce an increase in locomotor activity. Pretreatment with saline resulted in a significant decrease in locomotor counts compared to baseline (see Fig. 2.4; $p<0.05$).

Figure 2.4 Effect of SB242084 (1mg/kg), MDMA (3mg/kg) and saline treatment on total locomotor activity. Rats were pretreated with either SB242084 or saline 30 minutes prior MDMA or saline self-administration. Data are presented as mean change from baseline over the 30-min test period (± SEM) for SB242084 + MDMA (n=7), Saline + MDMA (n=7), and SB242084 + Saline (n=4). No significant differences were observed for any group. *p<0.05, **p<0.01 denotes significant effect compared to baseline.

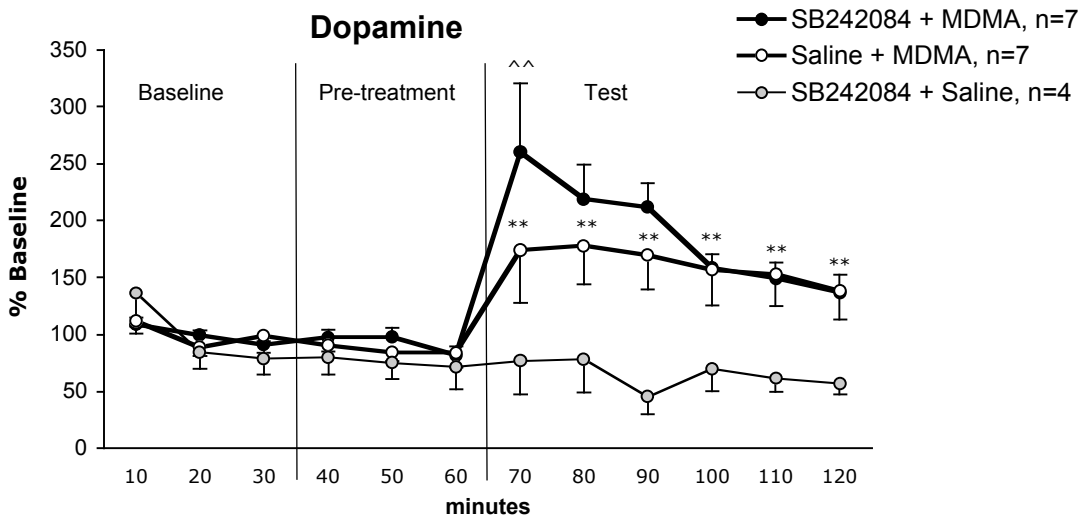


**Effect of drug treatment on nucleus accumbens DA and 5-HT release**

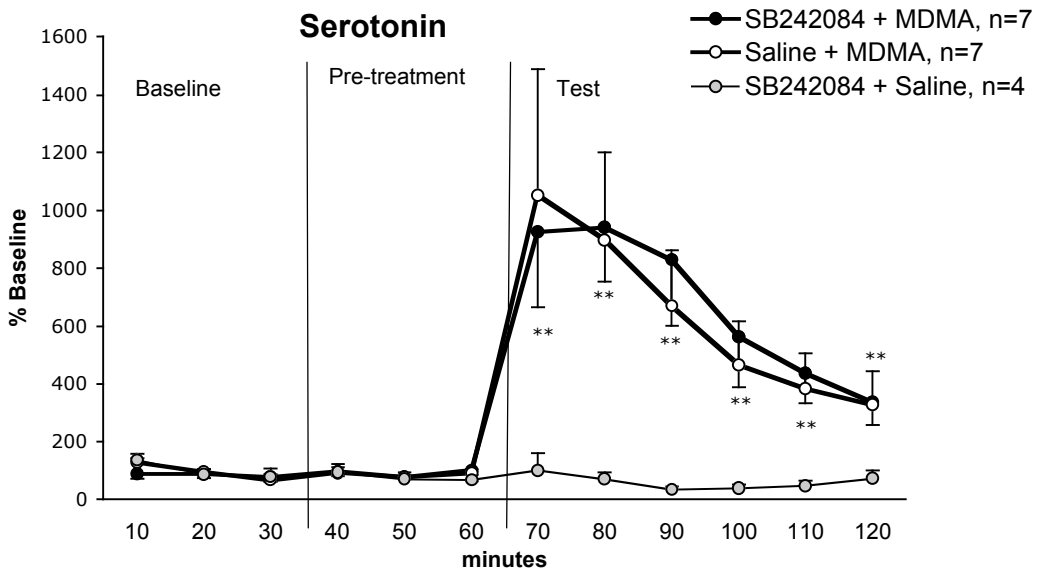
MDMA self-administration caused a significant increased in DA and 5-HT release in the nucleus accumbens (see Fig 3.4A and B). The two-way repeated measure ANOVA revealed a significant group effect ($F(2,15)=6.04$; $p=0.0117$), time effect ($F(11,165)=6.77$; $p=0.0001$), and group X time interaction ($F(22,165)=3.34$; $p=0.0001$) for DA release. Similarly, the two factor repeated measures ANOVA showed a significant group effect ($F(2,15)=3.63$; $p=0.05$), time effect ($F(11,165)=11.01$; $p=0.0001$), and group X time interaction ($F(22,165)=3.04$; $p=0.0001$) for 5-HT release in the Nacc. However, 5-HT release was more pronounced (approximately 1000x fold of baseline at time point 70min) for both MDMA groups compared to MDMA-mediated DA release (approximately 200x fold). Post-hoc analyses showed pre-treatment with SB242084 elicited a significant enhancement of MDMA-induced DA ($p<0.01$; see Fig. 3.4A), while no further increase in extracellular 5-HT was appreciated (see Fig. 3.4B).

Figure 3.4 Effect of SB242084 (1mg/kg), MDMA (3mg/kg) and saline treatment on extracellular levels of DA and 5-HT in the NAC. Rats were pretreated with either SB242084 or saline 30 minutes prior MDMA or saline self-administration. A) Data are expressed as the percentage change from baseline DA (A) or 5-HT (B) levels (mean±SEM) for each 10 min bin for the 120 min total session period, for SB242084 + MDMA (n=7), Saline + MDMA (n=7), and SB242084 + Saline (n=4). Mean extracellular basal levels of DA (pg/$\mu$l): 0.58 ± 0.08, 0.73 ± 0.17, and 0.75 ± 0.21 for SB242084 + MDMA, Saline + MDMA, and SB242084 + Saline respectively. Mean extracellular basal levels of 5-HT (pg/$\mu$l): 0.12 ± 0.02, 0.13 ± 0.06, and 0.11 ± 0.05 for SB242084 + MDMA, Saline + MDMA, and SB242084 + Saline respectively (values corrected for recovery of the dialysis probe). ** = significant differences between MDMA and saline self-administration at $p < 0.01$. ^^ = significant differences between groups post-MDMA administration at $p<0.01$ (Fisher's LSD).

A


Dopamine
SB242084 + MDMA, n=7
Saline + MDMA, n=7
SB242084 + Saline, n=4
Baseline
Pre-treatment
Test
^^
**
% Baseline
minutes
0
50
100
150
200
250
300
350
10
20
30
40
50
60
70
80
90
100
110
120

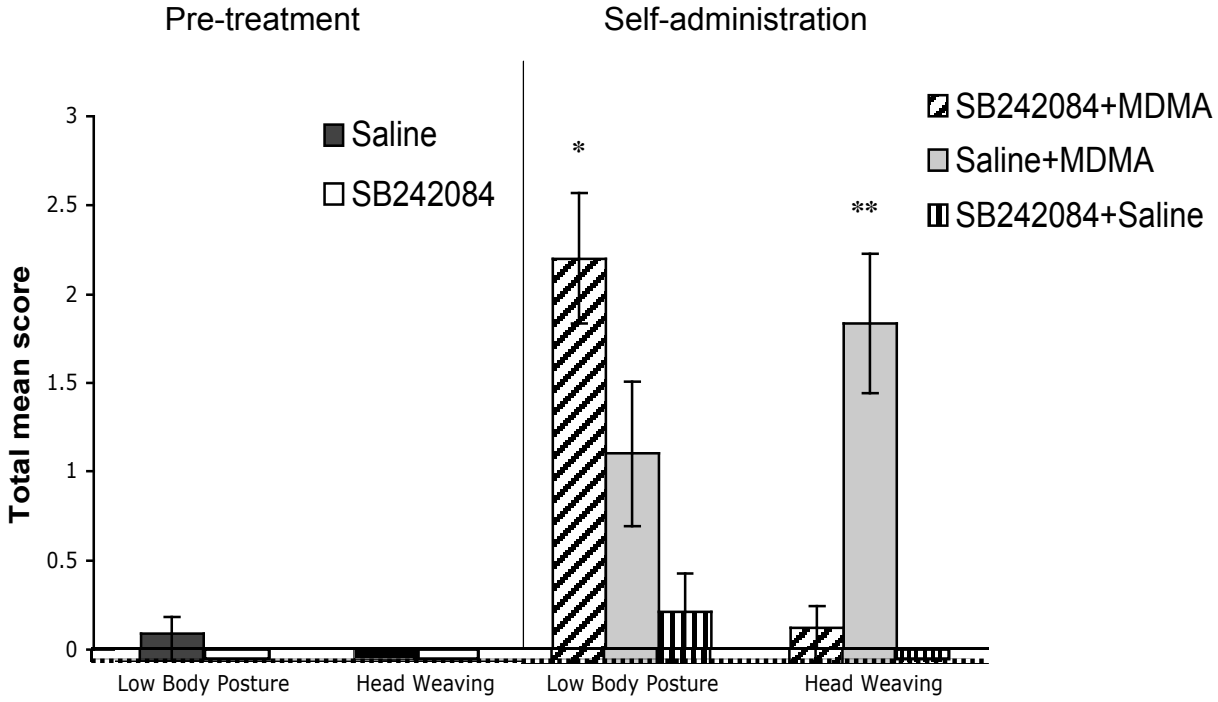
B


Serotonin
SB242084 + MDMA, n=7
Saline + MDMA, n=7
SB242084 + Saline, n=4
Baseline
Pre-treatment
Test
**
% Baseline
minutes
0
200
400
600
800
1000
1200
1400
1600
10
20
30
40
50
60
70
80
90
100
110
120

**Effect of drug treatment on specific behaviors**

Low body posture behavior analyzed by two-way repeated measures ANOVA showed the effect of treatment ($F(2,15)=9.45$; $p=0.0022$), time ($F(1, 15)=18.41$; $p=0.0006$), and interaction between treatment and time ($F(2,15)=4.17$; $p=0.0362$). The two-way repeated measures ANOVA for head weaving behavior showed a significant drug effect ($F(2,15)=12.41$; $p=0.0007$), time effect ($F(1, 15)=15.05$; $p=0.0015$), and drug X time interaction ($F(2,15)=12.41$; $p=0.0007$). Post-hoc analysis showed MDMA self-administration (3mg/kg) induced low body posture behavior-movement in SB242084 pretreated animals compared to saline pretreated rats ($p<0.05$; see Fig. 4.4). Contrarily, pre-treatment with saline significantly induced head weaving behavior after MDMA self-administration compared to SB242084 pre-treated rats ($p<0.01$; see Fig. 4.4). SB242084 pre-treatment alone did not induce any of these behaviors.

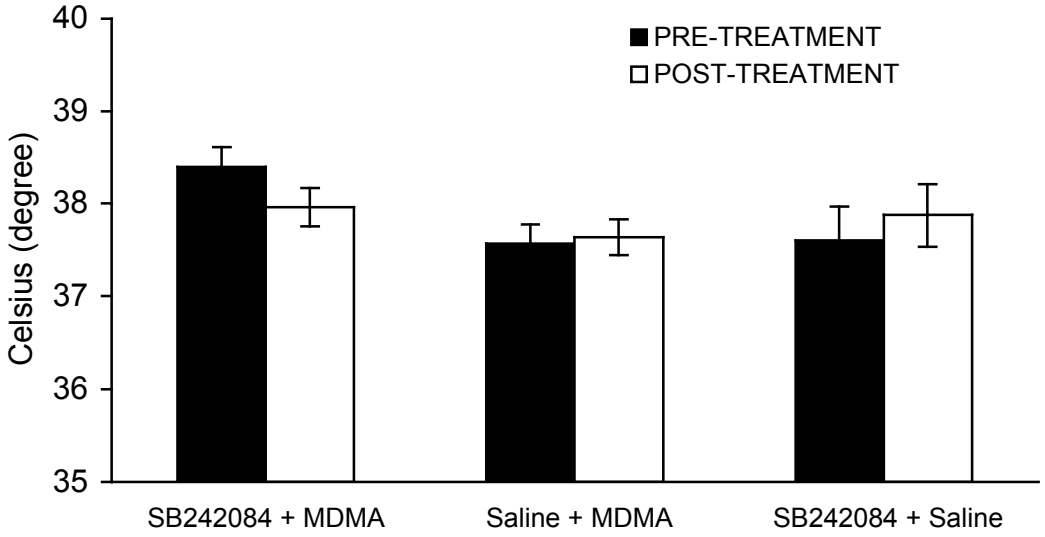
Figure 4.4 Effect of SB242084, saline, and MDMA treatments on specific behaviors of the serotonin syndrome. Data represents the total mean score ± SEM for low body posture ( ■ ) behavior, and head weaving ( □ ) behavior for SB242084 (1mg/kg) or saline pretreatment and MDMA (3mg/kg) or saline self-administration.



**Effect of drug treatment on rectal temperature**

Our results revealed none of the different drug combinations caused a significant effect on rectal temperature (see Fig. 5.4).

Figure 5.4 Effect of SB242084 (1mg/kg), MDMA (3mg/kg) and saline treatment on rat rectal temperatures. Rats were pretreated with either SB242084 or saline 30 minutes prior MDMA or saline self-administration. Each value represents the mean ± SEM for SB242084 + MDMA (n=7), Saline + MDMA (n=7), and SB242084 + Saline (n=4). No significant differences were observed for any group.



## DISCUSSION

It is well established moderate to large experimenter-delivered doses of MDMA induce an increase in locomotor activity in rodents (Callaway et al., 1992; Gold and Koob, 1989; O'Shea et al., 2005; Spanos and Yamamoto, 1989). However, the results from the present study demonstrate self-administration of a moderate amount (3mg/kg) of MDMA did not induced hyperactivity in rats (see Fig. 2.4 and 4.4). A state of stress in known to induce an increase in extracellular DA in the NAcc of experimental animals (Abercrombie et al., 1989; Doherty and Gratton, 1997; Stevenson et al., 2003), and increased locomotor activity to psychostimulants has been previously associated with enhanced DA transmission (Chiueh and Moore, 1975; Hurd and Ungerstedt, 1989; Ritz et al., 1987; Seiden et al., 1993). Hence, these discrepancies might be explained by the fact that stress associated with involuntary drug administration might induce an increase in basal DA and locomotor response. Confirming previous findings (Kennett et al., 1997) we observed SB242084 (1mg/kg) alone did not caused hyperactivity. However, we observed a marked increase in locomotor activity when MDMA was paired with SB242084 compared to baseline (see Fig. 2.4).

Both 5-HT and DA actions on specific receptors have been linked to MDMA changes in behavior (Bankson and Cunningham, 2001; Bubar et al., 2004; Fletcher et al., 2002). Consistent with the literature (Colado et al., 1999; Gough et al., 1991; Gudelsky and Nash, 1996; O'Shea et al., 2005) MDMA treatment induced an increase in both DA and 5-HT in the NAcc (see Fig. 3.4A and B). Although *in vitro* studies suggest MDMA is a more potent 5-HT releaser (Crespi et al., 1997; Johnson et al., 1986; Nichols et al., 1982; Schmidt et al., 1987). *In vivo* experiments had revealed moderate doses of injected MDMA had a more pronounced effect on extracellular DA compared to 5-HT in the

NAcc (O'Shea et al., 2005; White et al., 1994). Our data indicate voluntary MDMA intake elicits a preferential increase in 5-HT than DA release in the NAcc (see Fig. 3.4A and B).

The information currently available suggests MDMA-induced hyperactivity might be in part modulated by excitatory and inhibitory actions of 5-$HT_{2A}$ and 5-$HT_{2C/B}$ receptors respectively (Ball and Rebec, 2005; Bankson and Cunningham, 2002). While previous studies have shown SB242084 interaction with 5-$HT_{2C}$ receptors mediates DA release preferentially in the prefrontal cortex (Gobert et al., 2000; Millan et al., 1998; Pozzi et al., 2002). One study found SB242084 (1mg/kg) to be a weak DA releaser (De Deurwaerdere et al., 2004) compared to mixed 5-$HT_{2B}$ /5-$HT_{2C}$ antagonist (SB206553) in the NAcc, while another study demonstrated 10mg/kg SB242084 failed to increase levels of these DA nor 5-HT in the same brain region (Gobert et al., 2000). Our data indicate SB242084 in combination with saline did not induced changes in extracellular DA or 5-HT release (see Fig 3.4). However, SB242054 pre-treatment significantly enhanced MDMA-mediated DA release and induced an increase in total locomotor counts compared to baseline (see Fig. 3.4A and Fig 2.4). In addition, SB242084 caused an increase in low body posture with movement behavior compared to animals pre-treated with saline (see Fig. 4.4). These results are in line with a recent study (Navailles et al., 2004) that showed SB242084 ability to enhance cocaine stimulatory effects as well as NAcc DA release, and thus confirm the important role of 5-$HT_{2C}$ in the mechanism of action of many psychostimulants. The results from this study offer corroborative support to the hypothesis that MDMA-associated hyperactivity might be in part mediated through inactivation of 5-$HT_{2C}$ receptors with the consequent increase in DA transmission. Hence, inhibitory action of 5-$HT_{2C}$ receptors might explain the lack of hyperlocomotion observed

after moderate dose (3mg/kg) MDMA self-administration. Although less is known about MDMA binding to 5-HT receptors, experimental studies suggest MDMA might bind to 5-$HT_{2A}$ receptors, and reduce their expression in rats and humans (Reneman et al., 2002; Scheffel et al., 1992). MDMA is also known to bind, although with less affinity that 5-HT, to 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors (Nash et al., 1994). Accordingly, direct MDMA binding to 5-$HT_{2C}$ receptors or increased extracellular 5-HT action on 5-$HT_{2C}$ receptors might be involved in the lack of locomotor response found in this study.

Many of MDMA effects are influenced by small changes in ambient temperature. For example, when animals are kept in warmer environments (30°C) MDMA-mediated increase in core temperature, accumbals DA release, and locomotor activity are more pronounced (O'Shea et al., 2005). In addition, small changes in room temperature that alter rectal temperature influence MDMA neurotoxic actions (Malberg and Seiden, 1998). The results from this work demonstrate rectal temperatures were no altered by any of the different drug treatments (see Fig. 5.4). Hence, any observed change in lomotor behavior or extracellular neurotransmitters levels are not the result of drug-induced increase in body temperature.

In conclusion, our study demonstrate 3-mg/kg MDMA self-administration has no locomotor activating properties. However, the same dose in combination with SB242084 (1mg/kg) pre-treatment induced an increase in locomotor activity and significantly enhanced extracellular DA levels in the NAcc. It has become more accepted the role of 5-$HT_{2C}$ as modulators of DA neurotransmission. However, much less is known about the importance and specific role of 5-$HT_{2C}$ receptors in MDMA actions in the brain. Our

study demonstrates 5-HT or MDMA effect on this receptor subtype might underlie some of the neurochemical and behavioral responses observed upon MDMA exposure.

## CHAPER 4 SUMMARY

Multiple 5-HT receptors have been associated with the acute effects of MDMA.

In this last study, we investigated the specific role of 5-$HT_{2C}$ receptors in MDMA-induced behavior, and DA and 5-HT release in the NAcc. Our results showed pre-treatment with SB242084 (a 5-$HT_{2C}$ inverse agonist) modified some of MDMA-associated 5-HT syndrome behaviors, and induced changes in MDMA-mediated NAcc DA release.

In summary, SB242084 or MDMA alone did not induce an increase in locomotor activity. In addition, SB242084 plus saline self-administration had no effect on NAcc DA or 5-HT levels assessed by *in vivo* microdialysis. However, the combination of SB242084 pre-treatment and MDMA (3mg/kg) self-administration caused an enhancement of MDMA-mediated NAcc DA release. Likewise, the low body posture behavior (with no net movement) characteristic of MDMA exposure was significantly attenuated, while a low body posture-movement behavior developed. An important characteristic of drugs that act on the dopaminergic system is their ability to induce an increase in locomotor activity. The results from this work demonstrate the positive correlation between enhanced NAcc DA activity and increased net motor behavior.

Given the widespread use of MDMA, additional information on the basis of MDMA addictive behavior is needed. An important approach to understand the reinforcing properties of MDMA would be to assess the behavioral and neurochemical changes that occur after long-term MDMA self-administration in SB242084 pretreated animals. It would be of interest to study alterations in MDMA lever responding with SB242084 pretreatment. In addition, substantial evidence link 5-HT receptors with

MDMA-mediated changes in behavior. Hence, a comprehensive behavioral analysis with MDMA experience would provide insights into the brain molecular targets necessary for MDMA reward. The results from this and similar studies, using various 5-HT receptors agonists/antagonists might shed light on mechanisms of voluntary MDMA consumption.

# GENERAL DISCUSSION

There are still many questions unanswered regarding MDMA pharmacology and mechanisms of toxicity in experimental animal. Several hypotheses have been proposed to explain the differences in MDMA actions in rats and mice. Still, the majority of neurotoxicity studies had focused on the 5-HT system and while some works have investigated MDMA toxic actions on mouse DA system, laboratory conditions, strains, or age differences in these studies have made the results difficult to interpret. The first part of this dissertation examined the age dependent sensitivity to MDMA in mice following a neurotoxic dose regimen. Consistent with previous works, our results show MDMA selectively affects the DA system, while no changes in 5-HT, or its metabolite were detected a week after treatment (see Table 1.1). Likewise, MDMA exposure results in long-term reductions in striatal VMAT2 expression in older animals, a stable marker of DA nerve terminal integrity (see Fig 4.1). Findings from this study revealed a significantly higher hyperthermic response in older mice compared to younger animals (see Fig. 2.1). Accordingly, we concluded that MDMA-induced increase in body temperature plays an important role in MDMA associated toxicity, but recognized other factors might be involved.

Stone and colleagues (Stone et al., 1987) revealed rats are more vulnerable to MDMA toxicity compared to mice, and the literature suggest dissimilar MDMA metabolic pathways between these two species might account for this difference. While no studies have been performed to compare striatal and hippocampal MDMA concentrations in mice and rats, findings from this study show a higher dose, and repetitive injections, are needed to deliver a similar amount of the parent drug into the

striatum (see Fig. 6.1) compared to the amount detected in rat hippocampal dialysate (Esteban et al., 2001). Thus, if extracellular MDMA alone, or in combination with increased DA release are responsible for MDMA DA axonal damage, the higher hippocampal concentrations found in rats might explain why this species are more susceptible. Evidence, however, suggest MDMA alone does not induce neurotoxicity in rats or mice when injected directly into the brain (Escobedo et al., 2004; Esteban et al., 2001; Paris and Cunningham, 1992). Esteban and collaborators (Esteban et al., 2001) found central injections of MDMA into the hippocampus induced a release in DA and 5-HT comparable to that observed when the drug is given parentally, but failed to cause hyperthermia. When rats were maintained in warmer rooms to mimic MDMA-associated hyperthermic response central perfusion of MDMA also failed to induce neurotoxicity in rats. A more recent study (Escobedo et al., 2004) aimed to investigate the neurotoxic effect of centrally administered MDMA in mice. The authors found direct injections of MDMA or HHMA, a major metabolite of MDMA in humans, into the mouse striatum did not cause long-term DA depletion. Similarly, MDMA nor HHMA failed to induce hyperthermia when injected directly into the brain. Hence, although the effect of central administration of MDMA on extracellular DA levels were not investigated, it is possible MDMA itself, in combination with increased body temperature, might be responsible for DA damage in mice. Taken together, these data suggest elevations in body temperature might exacerbate DA toxicity in mice, and is a major factor to consider in MDMA toxic effects in humans.

Scientists are starting to understand the human brain and the complex processes that underlie drug reward. Evidence suggests neurons in the brain are integrated to form neuronal pathways that modulate the activity of specific subpopulation of neurons. Drugs

of abuse, such as MDMA, work by interacting and changing the activity of different neuronal systems, including the DA and 5-HT system. Long-term exposure to MDMA triggers compensatory changes in key proteins that affect the brain neurochemistry, and hence behavior. Animal models of addition provide a tool to study the cellular and molecular mechanisms responsible for these long-lasting brain changes. In addition, changes in the animal behavior reflect neuroadaptational processes that are key to the development of drug addiction. For that purpose, in the second part of this dissertation, we examined the short- and long-term effects of moderate doses of MDMA using intravenous self-administration. A particular advantage of self-administration protocols is that researchers can study the behavioral and biochemical changes associated with voluntary drug intake and reinforcement. In addition, the rate of drug response is greater than when the drug is administered by any other route (i.e., subcutaneous (s.c.), intra-peritoneal (i.p.)). Initially, we confirmed optimal methods of obtaining MDMA self-administration behavior in rats. The acute and long-term effects of voluntary MDMA intake in rectal temperatures were also studied.

Inasmuch as most studies have shown MDMA exposure induces hyperthermia in rats, results from our study demonstrate moderate doses of self-administered MDMA caused a transient hypothermic response that was progressively reversed by day 10 (see Fig 2.2). As with the hypothermic response, our results demonstrate MDMA induces a massive release of NAcc 5-HT in naïve animals (see Fig. 6.3), kept under the same room temperature ($22\pm1$°C), thus this effect might be mediated through activation of 5-$HT_{1A}$ and 5-$HT_{2C}$ receptors (Blessing, 2004; Cryan et al., 2000). In fact, 5-HT plays a crucial role in thermoregulation, and administration of 5-HT elicits hypothermic responses in experimental animals (Hjorth, 1985; Sugimoto et al., 1991; Won and Lin, 1988). MDMA

is known to interfere with the mechanisms that regulate body temperature, but this study suggest thermoregulatory processes in the rat were able to compensate for the initial drop in rectal temperature. It is important to note, a progressive increase in locomotor activity, as well as induction of specific behaviors (i.e. piloerection) might have contributed to heat production. However, our results suggest other compensatory mechanisms involving up or down regulation of specific DA or 5-HT receptors, might be critical in the development of tolerance.

We were able to demonstrate MDMA, initially, induced a significant decrease in lever responses compared to animals that self-administered saline (non-reward). However, as MDMA intake increased, so did the number of lever presses for MDMA (see Fig. 2.3). By the end of the self-administration period, experienced MDMA animals showed significantly higher responses compared to days 1-10, and compared to saline animals by the end of the 20 days period. Interestingly, heat is a factor that is known to increase self-administration in rats (Cornish et al., 2003), thus (+/-)-MDMA induction of hypothermia during the first 8 days of self-administration might be responsible for the lower number of responses observed during this period.

Since DA and 5-HT release are thought to mediate the rewarding effect of drugs of abuse, we assessed MDMA-mediated neurotransmitter release in the NAcc of naïve animals and compared it to those of animals that voluntarily took (+/-)-MDMA for 20 days. Our results indicate the NAcc extracellular DA levels were significantly higher in naïve rats, while dialysis levels of 5-HT were comparable in both groups (see Fig. 6.3). The current consensus is that both monoamines might contribute to the rewarding properties of MDMA and other drugs of abuse (Bardo, 1998; Cole and Sumnall, 2003;

Daniela et al., 2004; Gold and Koob, 1988). For example, DA transporter knockout mice continue to self-administer cocaine (Rocha et al., 1998a), and mice lacking 5-$HT_{1B}$ receptors show increased cocaine self-administration (Rocha et al., 1998b). Hence, long-term changes in the ratio of extracellular NAcc DA/5-HT might be crucial for the development of MDMA self-administration and induction of drug-seeking behavior.

Research studies have suggested the crucial role of 5-$HT_{2C}$ receptors in MDMA pharmacology. For this reason, as part of this dissertation work, we aimed to study the actions of 5-$HT_{2C}$ receptors on MDMA-induced NAcc DA and 5-HT release and locomotor behavior. One major finding of that work was 5-$HT_{2C}$ inactivation was able to increase MDMA-mediated DA release and hyperlocomotion in animals that self-administered the drug (see Fig. 3.4, and 3.2). Serotonergic neurons innervate the DA system, and 5-HT neurotransmission has been shown to modulate DA release mainly via 5-$HT_{1B}$ (Cameron and Williams, 1994; Johnson et al., 1992) and 5-$HT_{2C}$ receptors (Benloucif and Galloway, 1991; Prisco et al., 1994). Several studies have implicated the 5-HT system in the facilitation of DA release *in vivo* via activation of various 5-HT receptors (e.g. 5-$HT_4$, 5-$HT_1$, 5-$HT_3$; Benloucif et al., 1993; Bonhomme et al., 1995; Galloway et al., 1993). In fact, dexfenfluramine, a potent 5-HT releaser, is thought to induce DA release through indirect 5-HT receptor activation (Balcioglu and Wurtman, 1998). More studies are necessary to investigate the effect of 5-$HT_{2C}$ blockage on long-term MDMA self-administration and reward. In this same study, animals were kept at a slightly lower room temperature, and a single 3mg/kg MDMA infusion did not induce hypothermia (see Fig. 5.4). This result indicates small variations in drug intake, and ambient temperature might influence MDMA effect on core temperature.

There are a myriad of toxicity studies that have investigated the effect of large doses of MDMA on the serotonergic and dopaminergic systems in rodents and non-humans primates. Far less research work has been conducted to study the rewarding properties of MDMA, or the long-term consequences of recreational amounts on the brain neurochemistry. Our results indicate post mortem tissue levels of 5-HT in the hippocampus (an area densely populated by 5-HT neurons), after 20 days MDMA self-administration sessions, were comparable to those of control animals 7 days after treatment (see Fig. 8.3A). Considering MDMA-associated increase in body temperature might contribute to its neurotoxic effects, the lack of hyperthermia observed in this study (see Fig. 3.3) might explain this result. While this study focused on MDMA-induced changes in extracellular DA and 5-HT levels in the NAcc, evidence suggest dopaminergic projections from the ventral tegmental area to the NAcc and PFC play a crucial role in the development of behavioral sensitization of drugs of abuse, including MDMA (Pierce and Kalivas, 1997; Ramos et al., 2005; Vanderschuren and Kalivas, 2000). Interestingly, long-last elevations of PFC DA levels were detected in the (+/-)-MDMA experienced animals (see Fig. 8.3G).

Taken together, these findings bring evidence MDMA chronic voluntary administration in rats induces long-lasting changes in brain regions that are associated with drug addiction and reward. The complexity of MDMA mechanisms of action arises from its interactions with various brain systems, including DA and 5-HT nerve terminals in the NAcc. MDMA human pattern of abuse should not be overlooked; hence more work is needed to investigate the effect of long-term MDMA self-administration on brain neurochemistry, and the behavioral changes associated with chronic MDMA exposure.

## SUMMARY OF DISSERTATION FINDINGS

MDMA age-dependent sensitivity in mice is poorly understood. Results from this dissertation have provided evidence the higher degree of hyperthermia observed in older mice might be critical in MDMA-mediated neurotoxicity observed in this age group. Since hyperthermia is known to potentiate MDMA-induced toxicity, age-related differences in the mechanism of heat regulation might explain the lower vulnerability observed in young mice. A better understanding of the mechanisms of hypergenesis induced by MDMA might be relevant for the development of new agents that provide protection against MDMA neurotoxicity.

The MDMA self-administration animal model used in this dissertation provides a tool to reliably study the mechanisms of MDMA reward and craving. Our results have shown self-administration of moderate doses of MDMA induce physiological, neurochemical and behavioral changes that are altered with MDMA experienced. Tolerance to the initial hypothemic respose and behavioral sensitization (e.g. total locomotor activity, and low body posture serotonin behavior) were detected with repetitive MDMA intake. In addition, chronic MDMA self-administration attenuates the efficacy of a single infusion of 3 mg/kg MDMA to increase extracellular levels of NAcc DA. However, tissue levels of PFC and NAcc DA were increased in MDMA experienced animals and NAcc 5-HT levels were reduced 7 days after the last MDMA infusion.

Results from this dissertation also provide evidence of the role of 5-$HT_{2C}$ receptors in the locomotor stimulant properties of MDMA. Likewise, blockage of 5-$HT_{2C}$ stimulation with SB242084 pre-treatment caused an enhancement in MDMA-mediated

NAcc DA release, while levels of extracellular NAcc 5-HT were comparable to those of saline pre-treated animals.

Overall, the findings from this dissertation work are a unique contribution to our understanding of the mechanisms of MDMA neurotoxicity in mice, and the changes associated with chronic MDMA self-administration in rats.

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# Vita

Maria Elena Reveron was born on October 1st, 1970 in Santa Cruz de Tenerife, Spain. She is the first-born child of Luis Reveron Garcia and Maria de los Angeles Castello Gomez, and she has two sisters. She majored in Pharmacy in June 1994 from the University of La Laguna, and she obtained a bachelor degree in Optometry in June 1995 from the University of Barcelona, Spain. She moved to the United States in summer 1998 to pursue her Ph.D. She obtained a M.S. degree at The University of Texas at Austin, for her work in the possible neurotoxic effect induced by prolonged L-DOPA exposure in mice, under Dr. Gary Miller's supervision. In Fall 2003, she joined Dr. Christine Duvauchelle's laboratory. Her dissertation work focuses on the behavioral and neurochemical changes associated with long-term MDMA self-administration in rats.

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