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Neuropharmacology of 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) and its stereoisomers

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# Neuropharmacology of 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) and its stereoisomers

By

Kevin Sean Murnane

B.S., University of Georgia, 2001

Advisor: Leonard Lee Howell, Ph.D.

An Abstract of
A dissertation submitted to the Faculty of the James T. Laney School of Graduate Studies of Emory University
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Abstract

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Racemic 3,4-methylenedioxymethamphetamine (MDMA) is a substituted phenethylamine that is widely abused as the street drug “ecstasy”. MDMA abuse is a high risk behavior that has been associated with severe deleterious consequences including acute lethality and brain changes indicative of long-term damage. MDMA produces complex biological effects consistent with a mixture of psychomotor-stimulant-like effects and hallucinogen-like effects. Previous studies have shown that the stereoisomers of MDMA may produce qualitatively different effects, suggesting a parsimonious mechanism for these complex effects. In the present experiments, we have sought to further explore the neuropharmacology of MDMA and its stereoisomers. In Chapter 2, we resolved some of the discrepancies and vagaries of the existing literature on the behavioral effects of MDMA by determining – using non-invasive measurements of sleep architecture and drug-discrimination – that the stereoisomers of MDMA engender qualitatively different behavioral and interoceptive effects. Furthermore, we determined that antagonism of the serotonin 5-$HT_{2A}$ receptor attenuates MDMA-elicited sleep disruption. In Chapter 3, we determined – using *in vivo* microdialysis and enzyme linked immunosorbent plasma analysis - that the stereoisomers of MDMA concomitantly elicited qualitatively different neurochemical and endocrine effects. Analogous to Chapter 2, additional experiments demonstrated some of these effects are attenuated by antagonism of the 5-$HT_{2A}$ receptor whereas others are attenuated by pretreatment with a selective serotonin reuptake inhibitor (SSRI). In Chapter 4, we determined – using functional magnetic resonance imaging (fMRI) – that the systems level neuropharmacological effects of MDMA and its stereoisomers are also qualitatively different. Collectively, this work strongly supports the hypothesis that qualitative differences in the effects of its stereoisomers mediate the complex biological effects of MDMA. Furthermore, this work supports the continued development of 5-$HT_{2A}$ receptor antagonists and SSRIs as novel pharmacotherapeutics for treating MDMA abuse. As such, these studies represent an important expansion of our understanding of the neuropharmacology of MDMA and its complex biological effects.

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# Chapter 1

## General Introduction

### History of MDMA

Racemic 3,4-methylenedioxymethamphetamine (MDMA) is a substituted phenethylamine that is widely abused as the street drug “ecstasy”. MDMA was first synthesized near the end of the nineteenth century. Previous authors have suggested that this synthesis was undertaken with the goal of developing a novel appetite suppressant. However, others have suggested that, as a point of fact, it was initially synthesized as a precursor for subsequent synthetic products and at the time it was not believed to have any interesting or important biological effects (Cohen, 1998; Green et al., 2003). Regardless of its purpose, it is clear that this initial synthesis led to few, if any, useful applications and did not result in widespread abuse of MDMA. For the next sixty years, MDMA remained in pharmacological obscurity until, in the 1950’s, the United States Army contracted a new synthesis. Following this synthesis, the structure activity relationship of mescaline derivative, including MDMA, toxicology was evaluated. MDMA was shown to have various dose-dependent effects in mice, rats, guinea pigs, dogs, and rhesus monkeys, including behavioral effects consistent with psychoactive properties such as anxiogenic and hallucinogenic effects in dogs and monkeys (Hardman et al., 1973). Again, this new synthesis led to few, if any, useful applications and did not result in widespread abuse of MDMA. This may be because the results of the program were not disseminated for 20 years. In the 1970’s, a new synthesis of MDMA was carried out and, unlike previous eras, this

time the effects of MDMA garnered considerable interest. The discernable difference in interest in MDMA subsequent to this synthesis may have been, at least in part, due to the clear psychoactive effects in humans.

At the time Dr. Alexander Shulgin synthesized MDMA, he had already become a recognized proponent of the use of hallucinogens for recreational and psychotherapeutic purposes. Dr. Shulgin had spent much of his life synthesizing a range of hallucinogen derivatives and determining their psychoactive properties by administering them to himself, his family, and his “volunteers”. Indeed, Dr. Shulgin eventually published an account of the psychoactive effects and instructions for carrying out the synthesis of literally hundreds of hallucinogenic derivatives in “Phenethylamaines I have known and loved: A chemical love story” (Shulgin, 1991). In his self named “clinicial trials” these “volunteers”, described as experts in the psychoactive effects of hallucinogens, wrote lengthy case reports on the subjective effects engendered by each derivative. The subjective effects of MDMA included an “altered state of consciousness with emotional and sensual overtones” (Shulgin, 1986). These effects had some similarity to those of other hallucinogenic phenethylamines but were also clearly different. Some of the more salient and unusual effects reported were increased empathy, communication, understanding, and feelings of closeness to others. Some psychotherapists suggested that these so called “empathogenic” effects supported the use of MDMA in counseling sessions (Greer and Strassman, 1985; Grinspoon and Bakalar, 1986; Greer and Tolbert, 1998). In one study, the authors concluded, following administration of MDMA to 29 patients in therapeutic sessions, that the

best use of MDMA was as an adjunct to “insight oriented” psychotherapy designed to facilitate communication and intimacy between couples and, somewhat ironically, as a treatment for abuse of alcohol and other drugs (Shulgin, 1986). Not long after, and perhaps not coincidentally, MDMA abuse began to increase at club parties or “raves”, initially under the street name “Adam” and later under the possibly more marketable name “ecstasy”. These parties were, and continue to be, all night affairs that tightly intermingle loud electronically generated music, unusual computer generated video and laser shows, extensive opportunities to engage in arrhythmic dancing, and heavy drug abuse. This likely provides a context conducive to the effects of MDMA.

While MDMA abuse was increasing, evidence was simultaneously growing that MDMA may have long lasting and pronounced deleterious effects in the brain. Two of the most widely cited studies of these “neurotoxic” effects showed MDMA or its major metabolite MDA depleted tissue content of serotonin in the rat (Ricaurte et al., 1985) and the squirrel monkey (Ricaurte et al., 1988). Further study showed that these serotonin depletions may be mediated through an axotomy or lesion of the terminal processes of serotonin projection neurons (O'Hearn et al., 1988). The combination of an increasing level of abuse, the lack of accepted safety for medicinal uses, and the growing evidence that MDMA may be neurotoxic led the United States Drug Enforcement Agency (DEA) to place MDMA into the most restricted category – Schedule 1 – of the Controlled Substances Act. This scheduling procedure itself produced considerable controversy, as the DEA circumvented typical scheduling procedures by invoking

emergency scheduling powers, and thereby circumvented judicial procedures wherein the psychotherapists could present evidence in regards to the safety of MDMA's use for medicinal purposes (Green et al., 2003). Putting aside the controversy of MDMA's drug scheduling, it has clearly not achieved its primary objective as significant abuse of MDMA has continued to this day.

## Epidemiology of MDMA abuse

After its appearance in the 1980s, abuse of MDMA has become widespread and prevalent in the last two decades. Epidemiology studies of drug abuse have often used survey based methodologies such as questionnaires or interviews which, without an extensive discussion of their limitations, function as an estimate of MDMA abuse. Subjects are typically recruited using advertisements and may be drawn randomly from the general population or from known drug abusing subpopulations. Estimates of the prevalence of drug abuse indicate that the abuse of most drug classes has remained fairly stable since the 1980s. However, abuse of MDMA and related derivatives has shown an unparalleled and rapid increase over the same time span. Survey estimates of a rapid increase in MDMA abuse have been corroborated by evidence of increased supply, demand, drug seizures, arrests of distributors – particularly arrests associated with organized crime -, and medical reports of adverse reactions (Rosenbaum, 2002; Green et al., 2003; Banken, 2004). The United States Department of Health and Human Services publishes three annual surveys of drug abuse trends. The 2007 Youth Risk Behavior Survey showed that 5.8% of

high school students had previously abused MDMA. The 2008 National Survey on Drug Abuse and Health found that 1.1 million Americans aged 12 or older had abused a hallucinogen – defined as psychadelic hallucinogens such as lysergic acid diethylamine (LSD) or psilocybin and "club drugs" such as Phencyclidine (PCP) and MDMA – in the previous *month*. The 2009 Monitoring the Future Survey showed that close to 5% of high school seniors has abused MDMA in the previous year (www.nida.gov). This was comparable to the prevalence of cocaine abuse (close to 4%) and approximately *five times higher* than the prevalence of heroin abuse (roughly 1%). Abuse of MDMA may be even more prevalent in certain subpopulations. Previous work has suggested that people that regularly attend "raves" or music clubs, college students, polydrug abusers, and Europeans may be particularly prone to MDMA abuse. One such study found that when United Kingdom respondents were recruited via dance music magazine advertisements, rather than randomly sampling the population, 96% had previously abused MDMA. This was a higher prevalence, in these subjects, than abuse of other amphetamines, marijuana, cocaine, or LSD. Furthermore, respondents reported on average abusing MDMA for the previous 4 – 5 years with a subgroup having abused MDMA for over a decade (Winstock et al., 2001). College students may also be particularly vulnerable to MDMA abuse as a randomly sampled survey of U.S. university undergraduates found that 39% had previously abused MDMA (Peroutka, 1987). Abuse of other illicit compounds may also be a risk factor for MDMA abuse. Indeed, when a sample was taken of known current drug abusers, 82% reported abusing MDMA within the previous

year (Williamson et al., 1997). Finally, it has been estimated that of all the illicit drugs available MDMA is the second most commonly abused drug, after cannabis, in a number of European countries (Schifano, 2004). Together, these studies provide strong evidence that certain subpopulations may be disproportionately susceptible to MDMA abuse.

Previous authors have penned apocryphal assertions that MDMA has a lower potential for addiction than other drugs of abuse. These assertions are based, in part, on the patterns of MDMA abuse and the lack of MDMA-elicited dependence (El-Mallakh and Abraham, 2007). In regards to whether or not MDMA produces dependence, it is important to recognize that, unlike opiates, psychomotor-stimulants, such as cocaine or amphetamine, do not produce physical dependence (JS Meyer, 2005; Meyer and Quenzer, 2005). Furthermore, the Diagnostic and Statistical Manual (DSM-IV) of the American Psychiatric Association lays out criteria, other than physical dependence, that can be used to diagnose drug dependence (APA, 2000). Using these DSM-IV criteria, recent work showed that 43% of a sample of known club drug abusers were dependent on MDMA (Cottler et al., 2001). Further study showed that, as would be expected from the effects of other drugs of abuse, both the severity of and the proportion of subjects experiencing MDMA dependence is a function of the magnitude and length of MDMA abuse (for a review see Leung and Cottler 2008). This suggests that MDMA has a greater potential to produce dependence and as a consequence addiction than may have been previously recognized.

Abuse of MDMA is a high risk behavior that may lead to significant untoward acute and persistent consequences. Acute adverse effects of MDMA include tachycardia, arrhythmias, hypertension, metabolic acidosis, cerebral haemorrhages, convulsions, coma, rhabdomyolysis, mydriasis, thrombocytopenia, disseminated intravascular coagulation, acute kidney failure, and death (Parrott, 2001; Schifano, 2004). Indeed, emergency room (ER) visits due to MDMA consumption have increased in parallel to MDMA abuse. ER mentions of MDMA were fewer than 500 in 1994 but greater than 5,500 in 2001, a greater than tenfold increase in less than a decade (Banken, 2004). The long-term consequences of MDMA are less clear but considerable evidence suggests that they may be severely deleterious. Exposure to MDMA or closely related derivatives has been associated with neural changes such as loss of brain content of monoamine neurotransmitters and catabolic enzymes (Wilson et al., 1996; Kish et al., 2000), loss of neurotransmitter regulating proteins (McCann et al., 1998; Reneman et al., 2001), or decreased basal brain metabolism (Buchert et al., 2001; Obrocki et al., 2002). Furthermore, these neural alterations have been linked to deficits on measures of attention, executive function, and learning and memory (Bolla et al., 1998; Hanson and Luciana, 2004; McCardle et al., 2004; McCann et al., 2007). The widespread prevalence and severe deleterious effects of MDMA abuse support the contention that the development of new treatments to minimize its abuse is a public health imperative.

*In vivo* pharmacology of psychomotor-stimulants

While abuse of MDMA is clearly an ongoing public health concern, its behavioral, interoceptive, and neuropharmacological effects are not fully understood. Previous work demonstrates that MDMA has effects in common with both psychomotor-stimulants and hallucinogens. A rational medications development effort for the treatment of MDMA abuse will likely require an understanding of the effects it shares with each drug class. Psychomotor-stimulants are widely subcategorized as the methylxanthines, tropanes, and the amphetamines (Hardman and Limbird, 2001; Meyer and Quenzer, 2005). Substances from these categories have been used for medicinal purposes and abused for hundreds, if not thousands, of years (Meyer and Quenzer 2005). In the United States, both use and abuse of cocaine and amphetamine became widespread in the latter part of the nineteenth century through the first decades of the twentieth century. Medicinal uses included local anesthetic effects and promotion of wafefulness, however, some psychotherapists suggested use in psychiatry may also be of benefit (Sulzer et al., 2005). Indeed, the noted psychoanalyst Sigmund Freud wrote that effects of cocaine consist of "exhilarating and lasting euphoria, which does not differ in any way from the normal euphoria of a healthy person" and that after consumption of cocaine "long-lasting intensive mental or physical work can be performed without fatigue, it is as though the need for food and sleep, which otherwise makes itself felt peremptorily at certain times of the day, were completely banished" (Freud 1885).

While Dr. Freud's account may not be an unbiased description of cocaine's effects, it is illustrative of many of the core effects of psychomotor-stimulants. These effects depend on many factors including drug dosage, drug history, concurrent drug use, and environmental interactions. Nevertheless, the behavioral effects of stimulants can be summarized as including mild to moderate motor excitement including stereotyped behaviors, sleep disruption, decreased feeding behavior with subsequent anorexia, violent behavior, and positive reinforcing effects (Meyer and Quenzer, 2005). The interoceptive or, in the case of humans, subjective effects of psychomotor-stimulants have also been categorized. Researchers working with human subjects have developed a number of questionnaires, such as the Profile of Moods States (POMS; McNair et al., 1971; Tancer and Johanson 2001) and Visual Analog Scales (VAS; Harris et al., 2002; Tancer and Johanson 2003), to assess the subjective effects of psychoactive substances. While there may be inherent limitations to this approach, such as direct drug effects on accuracy of responding, these scales are widely regarded as allowing for the systematic and reliable determination of drug-elicited subjective effects (Kelly et al., 2003). Psychomotor-stimulant-like subjective effects measured by these scales include: drug high, euphoria, feelings of closeness to others, energetic feelings, stimulation, increased feelings of sociability, anxiety, increased self-esteem, and feelings of grandiosity (Harris et al., 2002; Tancer and Johanson, 2003).

Psychomotor-stimulants encompass a broad range of substances with a diverse set of chemical structures and pharmacological effects. Nevertheless,

many of the behavioral and interoceptive effects of these compounds have been closely linked to the dopamine system. Classic studies have shown that the locomotor-stimulant effects elicited by low doses of these compounds (Kelly et al., 1975) or stereotyped locomotor behaviors elicited by high doses of these compounds (Creese and Iverson 1975) are attenuated by lesioning various portions of the striatum, a region that receives extensive and relatively selective dopaminergic innervations (Haber, 1986; Haber and Fudge, 1997; Haber and McFarland, 1999; Haber and Knutson, 2010). Analagous studies showed that pharmacological depletion of dopamine stores (Simon et al., 1995) or pretreatment with a dopamine receptor antagonist (Cabib et al., 1991) produced similar results. The positive reinforcing effects of the psychomotor-stimulants are thought to be a key element in their abuse liability and addictive potential. These effects also appear to have an important dopaminergic component. In one of the most widely cited studies of the effects of psychomotor-stimulants, a tight correlation was shown between the potency of a series of cocaine analogs to be self-administered and their affinities at the dopamine transporter (DAT; Ritz et al., 1989). This study has been supported by other work showing that selective DAT inhibitors function as positive reinforcers (Wilcox et al., 2002). These data lie in stark contrast to the effects of selective inhibitors of the other monomamine transporters, namely the serotonin (SERT) and norepinephrine (NET) transporters, as these compounds have not been found to be self-administered by laboratory animals nor do they exhibit appreciable abuse liability (Howell and Byrd, 1995; Howell, 2008).

Neuroimaging studies have been particularly useful in determining the neuropharmacology of psychomotor-stimulants (for a review see Howell and Murnane 2008). In one classic study, positron emission tomography was used to study the *in vivo* binding sites of the psychomotor-stimulant cocaine via chemical addition of a radioactive isotope of carbon. Although binding was somewhat heterogeneous, regions with high dopamine transporter expression exhibited high cocaine binding. Furthermore, cocaine binding was inhibited by pretreatment with a selective DAT but not a selective SERT nor a selective NET inhibitor (Fowler et al., 1989). Similar studies in human subjects corroborated these findings (Volkow et al., 1995). Further study revealed a direct relationship between cocaine induced self-reports of “high” on the VAS and the time course of striatal cocaine binding (Volkow et al., 1997). These results have been expanded upon by studies that determined the relationship between occupancy of the DAT and the behavioral and interoceptive effects of psychomotor-stimulants. In this work, DAT occupancies greater than 60% were required for humans to report positive subjective effects after administration of cocaine (Volkow et al., 1997) or methylphenidate (Volkow et al., 1999) or to maintain self-administration of cocaine (Wilcox et al., 2002) or dimethocaine (Wilcox et al., 2005) in rhesus monkeys. Neuroimaging studies of the brain activational effects of psychomotor-stimulants have also implicated the dopamine system in their effects. Using a radioactive isotope of oxygen, it has been shown that an acute bolus of cocaine increases blood flow in frontal cortical regions rich in dopaminergic innervation (Howell et al., 2001; 2002). Blood oxygen level

dependent (BOLD) functional magnetic resonance imaging (fMRI) in human subjects provided similar findings (Breiter et al., 1997; Kufahl et al., 2005; Kufahl et al., 2008). Further, in an impressive demonstration of the power and selectivity of this modality, the subregional temporal dynamics of the fMRI signal were shown to predict which subjective effects subjects reported on the VAS questionnaire (Breiter et al., 1997; Kufahl et al., 2005). Collectively, these studies strongly support the role of dopamine in the neuropharmacology of psychomotor-stimulants.

*In vivo* pharmacology of hallucinogens

The *in vivo* effects of MDMA also show considerable commonality with those effects traditionally ascribed to the hallucinogens. A thorough understanding of the effects of MDMA will likely require study of its hallucinogen-like effects. Many hallucinogenic drugs are derived from two broad chemical classes, the phenethylamines and the tryptamines. These classes contain the protypical hallucinogens: 3,4,5-trimethoxyphenethylamine (mescaline), N,N-dimethyl-4-phosphoryloxytryptamine (psilocybin) and lysergic acid diethylamide (LSD). However, as a drug category hallucinogens are typically accepted to encompass a larger range of pharmacological substances, with mechanisms of action as diverse as cannabinoid agonism (e.g., Δ9-tetrahydrocannabinol), N-methyl-D-aspartate (NMDA) antagonism (e.g., phencyclidine), muscarinic receptor antagonism (e.g., scopolamine), and κ opioid agonism (e.g., salvinorin A). Hallucinogenic compounds have been used by humans in traditional

indigenous rituals for thousands of years (Meyer and Quenzer 2005). However, the modern era of hallucinogen research and hallucinogen abuse is widely accepted to have begun with the discovery of the prototypical hallucinogen LSD, in the 1940's. Over the next 15 to 20 years, knowledge of profound psychoactive effects of LSD, and other related compounds, was largely confined to the governmental, medical, and scientific communities. However, the counter-culture movement of the 1960's anti-establishment agenda found commune with the use of hallucinogenic compounds. Many of the leaders of the movement proposed the use of these compounds for a variety of non-medical purposes. This led the government to tightly regulate the availability of these compounds and subsequently place many hallucinogens into the most restrictive level of the Controlled Substances Act, i.e. Schedule 1. Since the 1960s, abuse of these compounds has remained a significant public health concern.

LSD was initially synthesized in 1938 by Albert Hofmann, while he worked on novel analeptics derived from ergot alkaloids for Sandoz Pharmceuticals. LSD showed little promise as an analeptic and research was temporarily halted. Several years later however, Dr. Hofmann decided to reexamine the effects of LSD and therefore began a new synthesis. While carring out this new synthesis of LSD, Dr. Hofmann was overcome with a series of strange sensations and had to vacate his laboratory and return home. His description of this event illustrates some the core effects of hallucinogens. He wrote, "At home, I lay down and sank into a not unpleasant intoxicated-like condition, characterized by an extremely stimulanted imagination. In a dreamlike state, with eyes closed (I found the

daylight to be unpleasanly glaring), I perceived an uninterrupted stream of fantastic pictures, extraordinary shapes with intense, kaleidoscopic play of colors" (Hofmann 1979). Not quite believing that a chemical compound could produce such a profound experience, Dr. Hofmann, and some colleagues at Sandoz, intentionally consumed LSD on several other occasions. As can be gleamed from Dr. Hofmann's account, the interoceptive effects of hallucinogens are somewhat more amorphous that those of the psychomotor-stimulants. Nevertheless, similar to the stimulants, researchers have developed rating questionaires, such as the hallucinogen rating scale (HRS; Strassman et al., 1994; Tancer and Johanson 2003), to objectively measure the drug-elicited subjective effects of hallucinogens. Hallucinogen-like effects on these measures include changes in somathesia (changes in the body such as feeling physically detached), affect (changes in emotions such anxiousness or closeness to others), cognition (changes in thoughts such as new insights or feelings of insanity), perception (changes in somotosensory, auditory, and visual detection and/or processing), and volition (changes in attention and self-control).

Preclinical study of hallucinogenic drugs is challenging as many of their effects are unmeasurable in a non-verbal species. This problem has been compounded by limitations on the behavioral assays available. Although there are a paucity of studies, to date, hallucinogens do not maintain self-administration in laboratory animals under any known conditions (Poling and Bryceland, 1979). As many humans regularly self-administer hallucinogens, this is widely regarded as a false negative of self-administration procedures. As such,

preclinical study of the *in vivo* pharmacology of hallucinogens has been confined to using two widely accepted pre-clinical assays, i.e. (1) drug-elicited behavior, and (2) drug-discrimination (Ator and Griffiths, 2003). Basically an assay of drug-elicited behavior involves the administration of a drug and the measurement of some simple unconditioned behavior that is known to be mediated by a discrete number of receptor subtypes. In this regard, the drug-elicited head twitch response (Corne et al., 1963; Corne and Pickering, 1967) has been of great utility in hallucinogen research. Head twitching occurs in rodents spontaneously, but importantly is selectively increased in frequency by the administration of various hallucinogenic drugs (Peroutka and Snyder, 1981; Colpaert and Janssen, 1983; Green et al., 1983; Goodwin and Green, 1985; Darmani et al., 1990; Fantegrossi et al., 2004a). Pre-pulse inhibition (PPI) of the acoustic startle response is another drug-elicited effect of great utility to hallucinogen research. In this assay, the startling effects of a rapid and loud acoustic stimulus are diminished by a similar stimulus of lower intensity that closely temporally precedes the main stimulus. Hallucinogens disrupt the effectiveness of this pre-pulse through what is conceptualized to be an impairment of sensorimotor gating (for a review see Braff et al., 2001). While these assays do not exhibit much *prima facie* similarity to hallucinogenic effects in humans, they are reasonably selective for the effects of hallucinogens. In other words, the results of these studies allow one to make a reasonably accurate prediction as to whether or not a drug would produce hallucinogen-like effects in humans. As such, they exhibit high predictive validity. Drug-discrimination procedures have also been an

important component of preclinical hallucinogen research. The drug-discrimination assay compares a novel drug with another drug of known pharmacology in terms of the internal state produced by those drugs. In other words, a drug-discrimination procedure would be used to determine whether or not animal subjects report (on an operant lever) that the internal state produced by a test drug is similar to the one produced by a known hallucinogen, such as LSD. This is termed substitution. Although it is not possible to know upon which effect of a drug the animal is basing this "decision", there is a very tight correlation between results in drug-discrimination procedures and "subjective effects" in humans (Schuster and Johanson, 1988; Brauer et al., 1997). Furthermore, drug-discrimination procedures likely measure central nervous system effects. In support of this contention, it has been shown the cocaine methionide, a cocaine analog that does not cross the blood-brain barrier but shares peripheral effects with cocaine, does not substitute for cocaine (Witkin et al., 1991; Terry et al., 1994). Therefore, if an animal reports that a novel drug substitutes for a hallucinogenic training drug or that a known hallucinogen substitutes for an novel training drug there is a very high probability that the novel drug would be hallucinogenic in people. This assay is not limited to hallucinogens and has been widely implemented with stimulants (Schama et al., 1997; Martelle and Nader, 2009), opioids (Walker et al., 1997; Makhay et al., 1998), benzodiazepines (Rowlett and Woolverton, 2001; Licata et al., 2005), and other drug classes.

The use of drug-elicited effects and drug-discrimination procedures has

been immensely helpful in determining the neuropharmacology of hallucinogens. Considerable evidence from these and human studies have implicated serotonin as a key mediator of hallucinogenic effects. With the discovery of serotonin as a biologically active substance (Rapport et al., 1948) it was immediately obvious to chemists that LSD and serotonin were structurally similar. This led to the logical investigation of the serotonergic system as the critical mediator of hallucinogenesis. Early work focused on suppression of serotonergic activity (Gaddum, 1953; Gaddum and Hameed, 1954) and suppression of dorsal raphe firing (Aghajanian et al., 1968; Aghajanian et al., 1970; Haigler and Aghajanian, 1973; Aghajanian and Haigler, 1974). However, these early ideas were abandoned due to irreconcilable results when experiments were carried out across numerous compounds. More recent work on the mechanisms of hallucinogenesis has focused on the 5-$HT_{2A}$ receptor. This latest hypothesis primarily emerged from work involving drug-discrimination procedures which demonstrated that the interoceptive effects of both phenethylamines and tryptamines could be blocked by 5-$HT_2$ receptor antagonists such as ketanserin and pirenperone (Colpaert et al., 1982; Colpaert and Janssen, 1983). These results were supported by studies showing that selective 5-$HT_{2A}$ receptor antagonists block the HTR (Fantegrossi et al., 2005a; Fantegrossi et al., 2006), and the potency with which they do so is highly correlated with the antagonist's affinity for 5-$HT_{2A}$ receptors (Peroutka and Snyder, 1981; Ortmann et al., 1982). This body of work was compelling because it provided a common mechanism of action between the phenethylamines and the tryptamines. Perhaps the most

convincing demonstration of 5-HT2-mediated hallucinogenic effects was reported in a seminal paper published over twenty years ago (Sadzot et al., 1989). In these studies, an incredibly tight correlation (r=0.97) between affinity at 5-$HT_2$ receptors and hallucinogenic potency in humans was established. Finally, recent evidence suggests that, in addition to direct agonists, drugs that release serotonin, and as a consequence function as indirect agonists at the 5-$HT_{2A}$ receptor, can have hallucinogenic effects in humans (Tancer and Johanson, 2003; Johanson et al., 2006). This work provides a compelling mechanism of action to be more thoroughly explored by future research.

Neuroimaging techniques have been less frequently utilized in the study of the hallucinogens than in the study of the psychomotor-stimulants. However, the few studies that have been conducted provided important insights. In one such study, the binding of an LSD analog was shown to localize to frontal and temporal cortices (Wong et al., 1987). This distribution of binding is similar to the distribution of the 5-$HT_{2A}$ receptor (Burnet et al., 1995) and the distribution of binding of the selective 5-$HT_{2A}$ receptor antagonist M100907 (Hall et al., 2000; Kristiansen et al., 2005). Furthermore, this distribution of binding has extensive overlap with the distribution determined for radiolabeled hallucinogens (in this study LSD and DOI), using autoradiography (McKenna et al., 1989). Neuroimaging studies of the brain activational effects of hallucinogens have also implicated the serotonin system in their effects. Using a radioactive analog of glucose, it has been shown that an acute bolus of psilocybin increases brain metabolism in frontal, temporal, and cingulate cortex (Vollenweider et al., 1997).

These findings were subsequently corroborated in a study using double-blind and placebo controls (Gouzoulis-Mayfrank et al., 1999). In an fMRI study, the cerebral blood volume (CBV) response to acute PCP administration was attenuated by clozapine but not raclopride (Gozzi et al., 2007), suggesting a role for 5-$HT_2$ receptors in this response and supporting previous results showing that, in addition to NMDA receptor antagonism, PCP can function as a 5-$HT_2$ receptor agonist (Kapur and Seeman, 2002). Collectively, these studies support the role of the serotonergic system in the effects of hallucinogens.

Interoceptive and behavioral effects of MDMA

Although MDMA was placed into Schedule 1 of the Controled Substances Act, its behavioral effects do not clearly fit into traditional delineations of drugs of abuse. Specifically, the racemic mixture of MDMA appears to produce a complex set of biological effects that include both a stimulant-like and hallucinogen-like component (Shulgin, 1986; Harris et al., 2002). Due to the unusual nature of these effects, Nichols (Nichols, 1986) postulated that MDMA represented a new class of compounds categorized as “entactogens”. This new class includes MDMA and its related analogs methylenedioxyethylamphetamine (MDE) and methylenedioxyamphetamine (MDA). The precise mechanisms for these complex and unusual biological effects of the entactogens remain to be determined. A thorough understanding of these mechanisms would likely be of great utility in the development of treatments for MDMA abuse.

MDMA produces a variety of behavioral effects in laboratory animals. Many of these effects are consistent with those produced by psychomotor-stimulants or hallucinogens. For example, it is well established that MDMA elicits a stimulation of locomotor activity in rodents (Slikker et al., 1989; Spanos and Yamamoto, 1989; Callaway et al., 1990; McNamara et al., 1995; De Souza et al., 1997; Fantegrossi et al., 2003; Fantegrossi et al., 2004a; Acquas et al., 2007). It may be important to note, however, that the pattern of locomotor activity may differ from those observed after administration of more “pure” stimulants. Indeed, amphetamine administration results in increased locomotor activity that is generally fairly evenly distributed across the entire locomotor recording apparatus. In contrast, MDMA administration results in increased locomotor activity that is predominantly distributed to the periphery of the chamber (Gold et al., 1989; Callaway et al., 1990; McCreary et al., 1999). Another hallmark effect of the psychomotor-stimulants is the capacity to maintain self-administration. Most, if not all, psychomotor-stimulants are robustly self-administered by laboratory animals. However, it is important note that the maintenance of self-administration is not specific to the stimulants as drugs from other classes, such as the opiates or benzodiazepines, also maintain self-administration. Nevertheless, the results of self-administration studies remain germane to the present discussion as hallucinogens purportedly do not maintain self-administration (Poling and Bryceland, 1979). As such, MDMA has reinforcing effects that are consistent with stimulants as it is self-administered by laboratory animals under a variety of conditions (Beardsley et al., 1986; Lamb and Griffiths,

1987; Fantegrossi et al., 2002; Fantegrossi et al., 2004b; Lile et al., 2005; Wang and Woolverton, 2007; Banks et al., 2008). MDMA also produces effects in laboratory animals indicative of hallucinogenic effects. Given the nature of the effects of hallucinogens, these drugs are more difficult to study in preclinical models. Indeed, it is unknown if animals, other than humans, experience hallucinations. Nevertheless, as previously discussed, animal models have been developed that show high predictive validity for hallucinogenic effects. Consistent with the effects of "pure" hallucinogens, MDMA increases head-twitch responses in mice (Fantegrossi et al., 2004a). Furthermore, MDMA decreases PPI of acoustic startle in the rat (Mansbach et al., 1989). Drug-discrimination procedures have also been of enormous utility in the preclinical study of MDMA. A long series of studies has shown that MDMA shares interoceptive effects with stimulants and hallucinogens (Glennon et al., 1988; Oberlender and Nichols, 1988; Baker et al., 1995; Bondareva et al., 2005; Yarosh et al., 2007; Fantegrossi et al., 2009b). Furthermore, these effects have been consistently reported across a wide range of experimental conditions. Collectively, these studies demonstrate that MDMA shares effects with stimulants and hallucinogens in laboratory animals.

The effects of MDMA in clinical studies are largely analogous to those measured in preclinical studies. When administered MDMA under controlled laboratory conditions, human subjects report subjective effects that share properties with hallucinogens and psychomotor-stimulants. In one such study, Davison and Parrott (1997) wrote that recreational drug users reported MDMA-

elicited subjective effects consistent with stimulant effects such as elation, increased energy, and exhilaration. In addition, subjects reported effects consistent with hallucinogenic effects such as altered visual, auditory, and somatosensory perceptions. These results were corroborated by subsequent studies (Vollenweider et al., 1998; Liechti et al., 2000a). Further study demonstrated that these complex subjective effects were dose dependent and included other effects consistent with either stimulant-like or hallucinogen-like effects such as reports of drug high and LSD-like effects (Harris et al., 2002). These results were further extended by comparing the subjective effects of MDMA to the selective dopamine releaser amphetamine and the selective serotonin releaser meta-chlorophenylpiperazine (mCPP). Following administration of amphetamine, subjects reported, as expected, stimulant-like subjective effects. Following administration of mCPP, subjects reported hallucinogen-like subjective effects. MDMA shared subjective effects with each drug. In this elegant study, the researchers went on to determine the reinforcing and physiological effects of all three drugs. Amphetamine and MDMA produced both reinforcing and sympathomimetic effects whereas mCPP produced neither. This suggested that the common dopaminergic effects of amphetamine and MDMA, which mCPP lacks, mediate these reinforcing and sympathomimetic effects (Tancer and Johanson 2003). Finally, Tancer and Johanson recognized that the limits of individual reports of drug-elicited subjective effects may have particular relevance in the context of a drug that produces complex and unusual subjective effects. These procedures rely on the accuracy of the subject's

response which, in part, is determined by the subject's ability to accurately gauge which subjective effects they are experiencing. Therefore, they extended their findings by training subjects, using the principles of drug-discrimination, to reliably discriminate the interoceptive effects produced by amphetamine and mCPP. Even under these more stringent conditions, 50% of the subjects tested reported that MDMA-elicited interoceptive effects were similar to amphetamine whereas the other 50% of the subjects reported that they were similar to mCPP (Johanson et al., 2006). As a whole, this work provides compelling evidence that MDMA produces complex and unusual effects that share common features with both psychomotor-stimulants and hallucinogens.

## Mechanism of action of MDMA

The receptor and transporter pharmacology of MDMA was first studied by Battaglia and colleagues (1988). Using ex vivo radioligand binding, they determined that MDMA preferentially binds to a diverse set of proteins. However, this binding profile could be organized into subgroupings. At less than 1 µM, MDMA exhibited the highest affinity for the SERT. Between 1 and 10 µM, MDMA bound to $\alpha_2$ noradrenergic, 5-$HT_2$ serotonergic, M1 muscarinic, and H1 histaminergic receptors. Between 10 and 50 µM, MDMA bound to the DAT and NET and $\alpha_1$ noradrenergic, β noradrenergic, 5-$HT_1$ serotonergic, and M2 muscarinic receptors. MDMA bound to D1 and D2 receptors only at concentrations greater than 50 µM. Up to concentrations of 500 µM, MDMA

exhibited no appreciable affinity for acetylcholine transporters, opioid receptors, GABA receptors, or calcium channels, among others. Later study, using updated techniques, found similar results (Setola et al., 2003). However, these researchers were able to dissociate MDMA's binding at the three different 5-$HT_2$ receptors (i.e. 5-$HT_{2A}$, 5-$HT_{2B}$, and 5-$HT_{2C}$). Given the established role of the 5-$HT_{2A}$ receptor in hallucinogenesis it would have been reasonable to have expected that MDMA would have preferential affinity for this 5-$HT_{2A}$ receptor subtype. Surprisingly, MDMA exhibited the highest affinity for the 5-$HT_{2B}$ subtype. More recent work from our laboratory has extended these findings by showing that MDMA has appreciable affinity for the $\alpha_{2B}$ noradrenergic and 5-$HT_7$ receptors. Other recent work has characterized the interaction of MDMA at the biogenic amine transporters (Rothman et al., 2001; Rothman and Baumann, 2002) and the vesicular monoamine transporters (VMAT; (Partilla et al., 2006). The role of these proteins in the neuropharmacology of MDMA is not well understood in part due to their recent discovery and the lack of available pharmacological tools that selectively target these proteins. As such, the binding profile of MDMA can be summarized as complex but shows particular relevance for monoaminergic systems.

Functional *in vitro* studies have also implicated the monoaminergic systems as a key component of the neuropharmacology of MDMA. Early mechanistic studies of MDMA showed that it stimulated neurotransmitter efflux from vesicles that expressed the SERT and were preloaded [$^3$H]5-HT (Nichols et al., 1982; Johnson et al., 1986). Further study demonstrated similar releasing

effects on dopamine (Schmidt et al., 1987) and norepinephrine (Rothman et al., 2001). Concurrent work demonstrated that the monoamine releasing effects of MDMA, in rat brain, are mediated via non-exocytotic mechanisms (Berger et al., 1992; Fitzgerald and Reid, 1993; Crespi et al., 1997). Other researchers dissociated MDMA's effects at plasma membrane and vesicular transporters and demonstrated that MDMA is a substrate for both (Rudnick and Wall, 1992). This is a similar mechanism of action to amphetamine and quite distinct from the mechanism of action of cocaine. Considerable work with amphetamine (for a review see Sulzer et al., 2005) has demonstrated that what is now called "substrate based release" is mediated via two distinct effects. Drugs with this mechanism of action may bind to either vesicle bound transporters (such as the VMAT2) or plasma membrane bound transporters (such as the DAT). Upon binding, the drugs will reverse the normal function of either kind of transporter. In the case of a vesicle bound transporter, this will elicit release of neurotransmitter out of the vesicle and into the cytosol of the synaptic terminal. In the case of a plasma membrane bound transporter, this will cause the transporter to actively pump neurotransmitter out of the synaptic terminal and into the extracellular space, where it can bind to membrane receptors. Elegant work using COS cells expressing DAT, VMAT2, or both has shown that these distinct effects are additive (Pifl et al., 1995). In other words, the combined effect on neurotransmitter release is greater than that produced by each effect. This may explain the general finding that substrate based releasers increase extracellular neurotransmitter levels to a greater extent than drugs that passively block the

reuptake of neurotransmitters, such as cocaine (for a review of the mechanism of action of MDMA see Green et al., 2003, Sulzer et al., 2005, Fleckenstein et al., 2007). MDMA binding at central receptors may modulate these neurotransmitter releasing effects or it may have direct effects in its own right. Further research is necessary to determine the underlying interaction between MDMA's effects at central transporters and receptors. However, it is clear that effects at monoamine proteins play a critical role in the neuropharmacology of MDMA.

Preclinical study has demonstrated a key role for both dopamine and serotonin in the behavioral and interoceptive effects of MDMA. For example, pretreatment with antagonists selective for either the D1 or D2 dopamine receptors attenuated the locomotor-stimulant effects of MDMA whereas its interoceptive effects were only attenuated by the D1 selective antagonist (Bubar et al., 2004). These neurobiological systems also have demonstrated relevance for the psychoactive effects of MDMA in humans. For example, chronic treatment with SSRIs, such as citalopram or paroxetine, attenuate the euphoric effects of MDMA (Stein and Rink, 1999). Further research demonstrated that acute pretreatment with citalopram attenuated many of the subjective effects of MDMA, supporting a serotonergic component in the psychobiology of MDMA (Liechti et al., 2000a). However, some of the effects of MDMA, such as emotional excitation, were unaffected by citalopram, suggesting that serotonergic effects do not fully account for its psychobiology. Additional work showed that citalopram, haloperidol (a mixed D2 dopamine/ 5-$HT_{2A}$ serotonin receptor antagonist), and ketanserin (a non-selective 5-$HT_2$ sertonin receptor antagonist) each attenuated

a distinct spectrum of MDMA's subjective effects (Liechti and Vollenweider, 2001). Specifically, 5-$HT_2$ receptor antagonism tended to attenuate the perceptual and emotional exciting effects of MDMA whereas D2 receptor antagonism tended to attenuate the euphoric and positive mood-state enhancing effects of MDMA. Collectively, this work demonstrates that dopaminergic and serotonegic pathways are critical mediators of the psychopharmacology of MDMA.

While MDMA's effects can be extensively organized into serotonergic and dopaminergic effects, it is important to recognize that there are established binding sites for MDMA outside of these systems. Furthermore, MDMA administration elicits pronounced endocrine effects that may have important psychobiological implications. For example, MDMA administration elicits secretion of oxytocin, cortisol, and prolactin (Harris et al., 2002). These hormones may mediate some of the subjective effects of MDMA, such as feelings of closeness to others, anxiogenic effects, and empathogenic effects. As such, the neuropharmacology of MDMA exhibits considerable complexity. Indeed, a tight correlation has been demonstrated between MDMA-elicit oxytocin secretion and prosocial feelings (Dumont et al., 2009). This complex set of effects led Parrot (2001) to describe the neurophamacological effects of MDMA as "a minefield of potential drug interactions."

The underlying mechanisms for the complexity of MDMA's psycho and neuropharmacological effects are not well understood. However, evidence suggests that it may be related to the unusual chemistry of MDMA. Chemically,

MDMA falls into the phenethylamine class of molecules. Collectively, this class contains a diverse set of compounds that range in their psychoactive properties from pretty “pure” stimulants, such as amphetamine, to pretty “pure” hallucinogens, such as mescaline, to mixed-action compounds, such as MDMA. All of these compounds share the basic phenethylamine ring structure, a chemical backbone that is synthesized by enzymatic decarboxylation of the amino acid phenylalanine (Fantegrossi et al., 2008). As such, the chemical structure of MDMA shares considerable overlap with these amphetamine-type stimulants and mescaline-type hallucinogens (Figure 1-1). Structure-activity relationship (SAR) studies of the phenethylamines have established that ring substituents, particularly by oxygen-containing groups, tend to facilitate hallucinogenic effects whereas amine additions tend to establish stimulant effects (Glennon et al., 1989). MDMA (Figure 1-1, A) contains both of these modifications as it is methylenedioxylated at the 3 the and 4 positions of its phenol ring and methylated at the amine. However, MDMA appears to violate principles established by other SAR studies. For example, it has been established that, while other additions may be compatible, amine methylation tends to abolish hallucinogenic effects (Anderson et al., 1978). Furthermore, among the hallucinogenic phenethylamines, it has been established that the R(-) stereoisomer is the active form (Shulgin, 1973; Nichols and Glennon, 1984). However, each stereoisomer of MDMA appears to be biologically active. Indeed, evidence suggests that not only is each stereoisomer biologically active but they

produce distinct effects. As a whole, this work suggests that the unusual and complex effects of MDMA may be related to its remarkable chemical properties.

Evidence that MDMA possesses stereoisomers with distinct biological effects has been particularly compelling in regards to the underlying mechanism of MDMA's complex effects. However, it is important to note that the stereoisomeric hypothesis of MDMA's effects is controversial as drugs with chiral centers overwhelmingly give rise to stereoisomers that engender similar biological effects. These stereoisomers may differ in the potency with which they produce these effects but the effects are the same. Nevertheless, several studies have shown that the stereoisomers of MDMA tend to induce qualitatively different effects (i.e., apparent efficacy differences), which is suggestive of a mechanism for its complex biological effects. In this regard, on a molecular level, Battaglia and De Souza (1989) provided results suggesting that S(+)-MDMA may have greater affinity for monoamine transporters whereas R(-)-MDMA may have higher affinity for post-synaptic receptors. More recent work demonstrates that this binding profile may have marked relevance for the psychoactive effects of these stereoisomers as, S(+)-MDMA has a "stimulant-like" profile, with an $EC_{50}$ for the dopamine transporter that is approximately 30 times greater than *R*(−)-MDMA (Setola et al., 2003). Furthermore, *R*(−)-MDMA is "hallucinogen-like" in its effects, possessing measurable affinity for the 5-$HT_{2A}$ receptor (Lyon et al., 1986) and acting as an agonist of this receptor, as it stimulates phosphatidyl inositol hydrolysis upon binding (Nash et al., 1994). At the systems level, only *S*(+)-MDMA increases dopamine neurotransmission in the striatum of Sprague-

Dawley rats (Acquas et al., 2007). This work extends older findings of stereoselective effects on 5-HT release *in vitro* (Nichols, et al., 1982). On a behavioral level, *S*(+)-MDMA, but not *R*(−)-MDMA, elicits hyperthermia and locomotor activity in mice (Fantegrossi et al., 2003) whereas only *R*(−)-MDMA induces head-twitch behavior in mice through direct agonism of the 5-$HT_{2A}$ receptor (Fantergrossi et al., 2005). Finally, the results of drug-discrimination experiments show that the interoceptive effects of these stereoisomers appear to be distinct and consistent with the hypothesis that S(+)-MDMA more readily functions as a psychomotor-stimulant whereas R(-)-MDMA more readily functions as a hallucinogen (Glennon et al., 1988; Baker et al., 1995; Murnane et al., 2009). Collectively, this work suggests a parsimonious mechanism for the complex biological effects of racemic MDMA and led Fantegrossi (2003) to suggest that “the challenge of unraveling the unique effects of MDMA on physiology and behavior in not likely to be overcome without further research into the distinct pharmacology of the MDMA enantiomers.”

## Summary and study aims

MDMA is one of the most commonly abused drugs in the world. MDMA abuse is a high risk behavior that has been associated with severe deleterious consequences including acute lethality and brain changes indicative of long-term damage. MDMA produces complex biological effects consistent with a mixture of psychomotor-stimulant-like effects and hallucinogen-like effects. Furthermore, there is currently no pharmacotherapeutic available for the treatment of MDMA

abuse. A better understanding the mechanisms that mediate its complex biological effects may aid the development of novel treatment strategies for MDMA abuse and its long-term deleterious consequences. To this end, in the present experiments, we have sought to further explore the neuropharmacology of MDMA. Previous studies have shown that the stereoisomers of MDMA may produce qualitatively different effects, suggesting a parsimonious mechanism for the complex effects of the racemic mixture. As such, particular emphasis in these studies was placed on comparing all three forms of MDMA. In Chapter 2, we set out to further study the behavioral effects of MDMA and its stereoisomers. These studies were designed to try to resolve some of the discrepancies and vagaries of the existing literature. Further studies were undertaken to evaluate the 5-$HT_{2A}$ receptor as a novel pharmacotherapeutic target for treating MDMA abuse. In Chapter 3, we determined the effects of MDMA and its stereoisomers on release of the monoamines dopamine and serotonin as well as secretion of the anterior pituitary hormone prolactin. Analogous to Chapter 2, additional experiments were undertaken to establish the role of the 5-$HT_{2A}$ receptor and the SERT in the neuropharmacology of MDMA. Finally, in Chapter 4, we determined the systems level neuropharmacological effects of MDMA and its stereoisomers, using functional magnetic resonance imaging (fMRI). These studies expanded upon the site-directed approach taken in Chapter 3 and extended the results of that chapter to a whole brain determination. Collectively, this work represents an important expansion of our understanding of the neuropharmacology of MDMA and its complex biological effects.

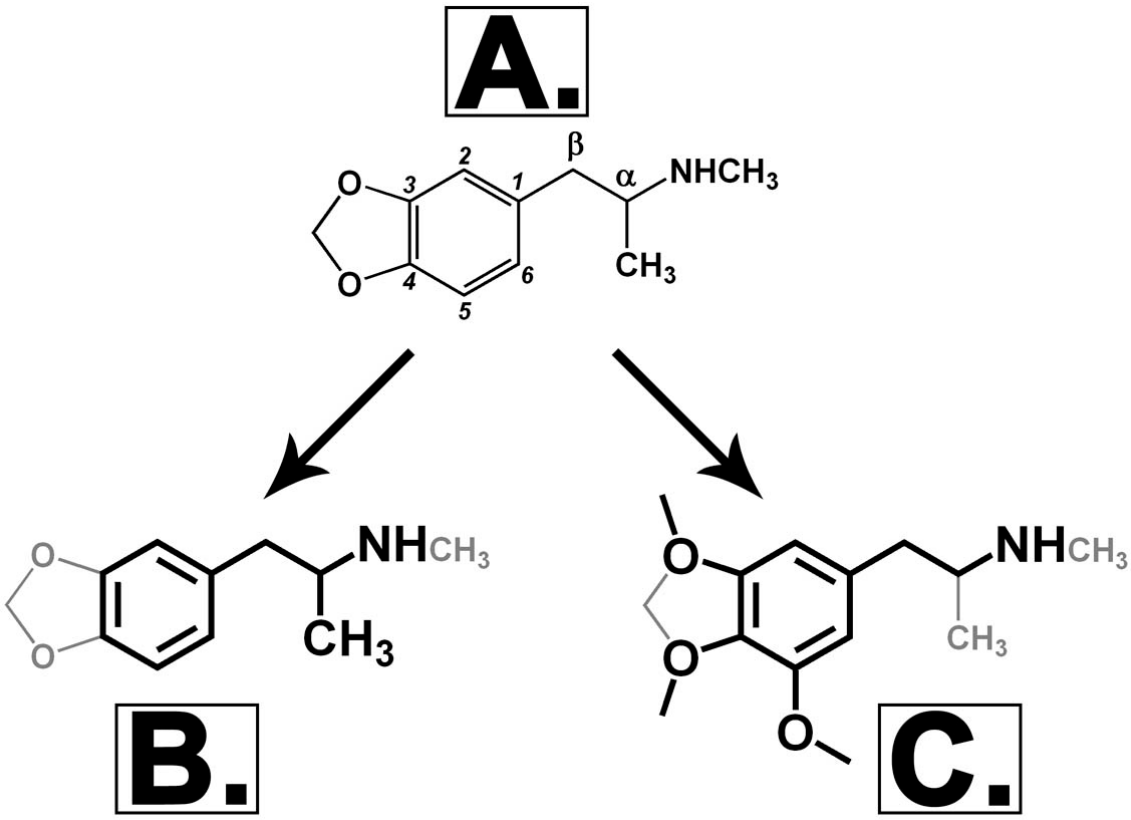


**Figure 1-1.**

Chemical similarities among MDMA, the amphetamine-type phenethylamine stimulants, and the mescaline-type phenethylamine hallucinogens. A, MDMA, with ring and side chain carbon position labels. B, amphetamine, bolded and overlaid on the structure of MDMA. C, mescaline, bolded and overlaid on the structure of MDMA.

# Chapter 2

## Interoceptive and behavioral effects of MDMA and its stereoisomers

Partially adapted from Murnane KS, Murai N, Howell LL, Fantegrossi WE "Interoceptive effects of psychostimulants and hallucinogens in S(+)-3,4-methylenedioxymethamphetamine (MDMA) and R(-)-MDMA trained mice." Journal of Pharmacology and Experimental Therapeutics 2009 Nov;331(2):717-23.

## Introduction

Racemic 3,4-methylenedioxymethamphetamine (MDMA) is a substituted phenethylamine that produces a complex profile of effects consistent with a mixture of psychomotor-stimulant and hallucinogenic effects. For example, human subjects report subjective effects such as “increased activation” and “heightened mood” (typical of psychomotor-stimulants), as well as “anxious ego-dissolution” and “oceanic boundlessness” (typical of hallucinogenic compounds). The precise mechanisms for these complex and unusual interoceptive properties of MDMA remain to be determined. However, several studies have shown that the stereoisomers of MDMA tend to induce qualitatively different effects (i.e., apparent efficacy differences), which is suggestive of a mechanism for its complex subjective effects. In support of this hypothesis, one of the earliest studies contrasted the subjective effects of these stereoisomers in humans (Anderson et al., 1978). The results of this report led to a series of studies utilizing drug-discrimination – the pre-clinical analogue of subjective effects in humans (Schuster and Johanson, 1988; Brauer et al., 1997) – designed to further elucidate any differences in the interoceptive effects of each stereoisomer.

Initial studies examining the stereoisomers of MDMA in the context of drug-discrimination were carried out concurrently by two distinct research groups. Glennon and colleagues (1988) reported that in rats trained to discriminate either the phenethylamine stimulant S(+)-amphetamine or the phenethylamine hallucinogen SR(+/-)-2,5-dimethoxy-4-methylamphetamine (DOM) from saline,

S(+)-MDMA fully substituted for the interoceptive cue produced by amphetamine but did not substitute for DOM. In studies conducted by Nichols and colleagues (1988), neither SR(+/-)-MDMA, S(+)-MDMA, nor R(-)-MDMA substituted for amphetamine in rats trained at the same training dose used by Glennon and colleagues (1988). The interpretation of these studies was that MDMA and its enantiomers produced an interoceptive cue that was distinct from either stimulants or hallucinogens. In later studies, rats were trained to directly discriminate each MDMA steroisomer from saline. Baker and colleagues (1995) found that the *S*(+)-MDMA cue partially generalized to phenethylamine stimulant *S*(+)-amphetamine and the tropane stimulant cocaine whereas R(-)-MDMA only generalized to cocaine. It is noteworthy that other results in this study were not supportive of *S*(+)-MDMA being a pure psychomotor-stimulant, because it also partially or fully generalized to the tryptamine hallucinogen mescaline and the ergoline hallucinogen lysergic acid diethylamine (LSD). More recent work from the Glennon group supports these findings as cocaine substituted in rats trained to discriminate S(+)-MDMA or R(-)-MDMA from saline (Bondareva et al., 2005). While some of these findings do not support the hypothesis that the stereoisomers of MDMA have qualitatively different effects, the preponderance of evidence across studies was supportive of distinct differences in the interoceptive effects of the isomers.

The stereoisomer mediated hypothesis of MDMA's complex effects has also been supported by behavioral studies using techniques other than drug-discrimination. MDMA reliably functions as a locomotor-stimulant in rodents

(Slikker et al., 1989; Spanos and Yamamoto, 1989; Callaway et al., 1990; McNamara et al., 1995; De Souza et al., 1997; Fantegrossi et al., 2003; Fantegrossi et al., 2004a; Acquas et al., 2007) and this effect is largely attributable to its S(+) stereoisomer in both mice (Fantegrossi et al., 2003) and rats (Acquas et al., 2007). Furthermore, through serotonin synthesis inhibition R(-)-MDMA has been shown to function as a direct agonist of the 5-$HT_{2A}$ receptor *in vivo* (Fantegrossi et al., 2005b). This is consistent with the known receptor pharmacology of hallucinogens. In nonhuman primates, consistent with psychomotor-stimulant-like effects, S(+)-MDMA and S,R(+/-)-MDMA, but not R(-)-MDMA, functioned as positive reinforcers under a progressive ratio schedule (Wang and Woolverton, 2007). In support of the previously described drug-discrimination literature, this further suggests that distinct effects of its stereoisomers may mediate the complex effects of racemic MDMA.

Despite these findings, other results call this hypothesis into question. In particular, studies utilizing nonhuman primates have yielded some results not supportive of this hypothesis. In one such study, MDMA did not engender robust increases in schedule controlled operant behavior when administered to squirrel monkeys working under a fixed-interval avoidance schedule (Fantegrossi et al., 2009a). These data are surprising as MDMA engenders robust and reliable locomotor-stimulant effects in rodents (Slikker et al., 1989; Spanos and Yamamoto, 1989; Callaway et al., 1990; McNamara et al., 1995; De Souza et al., 1997; Fantegrossi et al., 2003; Fantegrossi et al., 2004a; Acquas et al., 2007), humans report that MDMA produces stimulant-like subjective effects (

Vollenweider et al., 1998; Liechti et al., 2000; Harris et al., 2002; Tancer and Johanson 2003; Johanson et al., 2006), humans regularly engage in sustained locomotor activity subsequent to MDMA consumption (Tossmann et al., 2001; Green et al., 2003), humans report stimulant-like withdrawal symptoms subsequent to termination of MDMA abuse (Leung and Cottler, 2008), MDMA elicits sympathomimetic effects in humans (Vollenweider et al., 1998; Tancer and Johanson 2003), MDMA transiently relieves the motor deficits of Parkinsonian humans and MPTP treated squirrel monkeys (Colado et al., 2004), MDMA disrupts sleep in humans (Randall et al., 2009), and unlike hallucinogens, MDMA is self-administered by either baboons (Lamb and Giffiths 1987) or rhesus monkeys (Beardsley et al., 1986; Fantegrossi et al., 2002; Fantegrossi et al., 2004; Lile et al., 2005; Wang and Woolverton 2007; Banks et al., 2008). All of these effects suggest that there are many conditions under which MDMA functions as a psychomotor-stimulant. Nevertheless, other studies in nonhuman primates call into question the psychomotor-stimulant effects of MDMA. For example, in a study by Taffe and colleagues (2006) MDMA and its stereoisomers did not function as locomotor-stimulants. This further questions the robustness of MDMA's stimulant effects. Furthermore, the lack of any discernable difference between the various forms of MDMA does not support the generalization of the hypothesis that its stereoisomers have qualitatively different effects to this taxa. In addition, the profile of MDMA's effects across studies becomes even more murky when one considers the results reported by Fantegrossi and colleagues (2002; 2004). In these studies, all three forms of MDMA were found to be reliably

self-administered by rhesus macaques, under a fixed-ratio schedule. These results are also not supportive of qualitative differences between the stereoisomers of MDMA in this taxa, however, they lie in contrast to the lack of behavioral or locomotor-stimulant effects of MDMA previously described, as hallucinogens are purportedly not self-administered by laboratory animals (Poling and Bryceland, 1979). Furthermore, other results from these studies were supportive of qualitative differences between the stereoisomers of MDMA as pretreatment with the 5-$HT_{2A}$ receptor antagonist M100907 produced a parallel right-ward shift in the S(+)-MDMA self-administration dose-effect curve but a downward shift in the R(-)-MDMA curve (Fantegrossi et al., 2002). This suggests that while each stereoisomer was producing positive reinforcing effects they were producing these effects through different pharmacological mechanisms. The complexities and discrepancies in these studies suggests that further study of the psychomotor-stimulant effects of MDMA and its stereoisomers is warranted.

In the present experiments, we have attempted to try to resolve some the discrepancies in the drug-discrimination and psychomotor-stimulant literature of MDMA. The discrepancies between the results of published studies that examined the stereoisomers of MDMA using drug-discrimination may be related to the nature of the comparison compounds used in those studies. Across those previous studies, as the similarity of the chemical and pharmacological properties of the comparison compounds to MDMA increased so did the likelihood of finding a dissociation in the interoceptive effects of each stereoisomer. In the first set of experiments, the nature of the interoceptive cue engendered by *S*(+)- and *R*(−)-

MDMA was examined, in a parametric fashion in mice. Subjects were trained to discriminate *S*(+)-MDMA or *R*(−)-MDMA (1.5 mg/kg) from saline by using a two-lever, liquid food reinforced procedure. The generalization of each discriminative cue was then evaluated by full dose-effect determinations with substitution compounds that were parametrically varied in their structural and pharmacological similarity to MDMA (Figure 2-1): including the phenethylamine selective dopamine releaser *S*(+)-amphetamine (Davids et al., 2002) the nonselective tropane monoamine reuptake inhibitor cocaine (Davids et al., 2002), the phenethylamine 5-$HT_{2A}$ agonist 2,5-dimethoxy-4-(*n*)-propylthiophenethylamine (2C-T-7; Fantegrossi et al., 2005), and the mixed action tryptamine 5-$HT_{2A}$ agonist/serotonin reuptake inhibitor *N,N*-dipropyltryptamine (DPT; Blough et al., 2007). The specific hypothesis tested was that phenethylamine compounds that selectively share pharmacological effects with an isomer of MDMA would be more likely to substitute for the interoceptive effects of that MDMA isomer, and only of that isomer, in mice.

In the second set of experiments, the psychomotor-stimulant effects of MDMA were determined, in the context of sleep disruption, in rhesus macaques. As previously described, MDMA failed to exhibit robust behavioral-stimulant effects in squirrel monkeys responding under a fixed interval avoidance operant schedule (Fantegrossi et al., 2009) or locomotor-stimulant effects in rhesus macaques (Taffe et al., 2006). However, these data lie in contrast to considerable evidence suggesting that MDMA can function as a psychomotor-stimulant under a variety of conditions. It may be important to recognize that

MDMA abuse frequently occurs in the context of all night dance parties or “raves” (Rome 2001; Green et al., 2003). As such, the psychomotor-stimulant effects of MDMA may be more likely to be expressed as a disruption of sleep than as a change in daytime locomotor activity or schedule controlled operant behavior. In support of this contention, it has been shown that MDMA disrupts sleep in humans (Randall et al., 2009) and that some other psychomotor-stimulants more readily disrupt sleep than daytime activity (Anderson et al., in press). However, the acute effects of MDMA on sleep have been rarely studied. Furthermore, relatively few studies have examined the acute effects of any compound on sleep in rhesus macaques. Therefore, initial positive control experiments were undertaken to establish the effects of the known psychomotor-stimulant amphetamine in this species. These initial experiments were followed by full dose-effect determinations of the effects of MDMA and its stereoisomers. Finally, each compound was administered following pretreatment with the selective 5-$HT_{2A}$ receptor antagonist M100907 in order to determine if antagonism of this receptor would attenuate any sleep-disrupting effects of MDMA. The specific hypotheses tested were: 1) MDMA would disrupt sleep in rhesus macaques, 2) S(+)-MDMA would more effectively disrupt sleep than R(-)-MDMA, and 3) the sleep-disrupting effects of MDMA or either of its stereoisomers would be attenuated by antagonism of the 5-$HT_{2A}$ receptor.

## Materials and methods

### Subjects

Drug-discrimination studies were carried out in twelve (six per group) male Swiss-Webster mice (Charles River Laboratories, Inc., Wilmington, MA). Subjects weighed approximately 30 g and were housed three animals per 44.5 × 22.3 × 12.7 cm Plexiglas cage in a temperature-controlled room within the Yerkes National Primate Research Center. The rodent vivarium was maintained at an ambient temperature of 22 ± 2°C at 45 to 50% humidity, and lights were set to a 12-h light/dark cycle. Animals were fed Lab Diet rodent chow (Laboratory Rodent Diet 5001; PMI Feeds, Inc., St. Louis, MO) and water ad libitum until immediately before testing. Mice were not used in experiments until at least 5 days after arrival in the laboratory. Sleep studies were carried out in five female rhesus monkeys (*Macaca mulatta*) weighing between 7.0 and 8.5 kgs. Subjects were housed individually within a primate colony with continuous access to water and were fed daily in the early afternoon at a consistent time relative to experimental procedure. Their diet consisted of Purina monkey chow (Ralston Purina, St. Louis, MO) supplemented with fresh fruit and vegetables and food restriction protocols were not utilized. Ambient conditions within the colony were maintained at a temperature of 22±2°C and at 45-50% humidity; room lighting was set to a 12-h light/dark cycle with light period active from 07:00 to 19:00. All of the studies were carried out in accordance with the Guide for Care and Use of Laboratory animals as adopted and promulgated by the National Institutes of

Health, and experimental protocols were approved by the Animal Care and Use Committee at Emory University.

## Drugs

S(+), R(-), and S,R(+/-)-methylenedioxymethamphetamine, 2C-T-7, and amphetamine were supplied by the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC). M100907 and DPT were synthesized at the Laboratory of Medicinal Chemistry at the National Institutes of Diabetes, Digestive and Kidney Disorders at the National Institutes of Health (Bethesda, MD) and was a generous gift from Dr. Kenner C. Rice. Doses were calculated and are expressed as salts. All drugs were dissolved in 0.9% sterile saline, and administered intraperitoneally or intermuscularly in the drug-discrimination or sleep studies, respectively. Where possible, the doses of each compound were chosen on the basis of positive results, under similar conditions, from peer-reviewed literature.

## Procedure

### *Drug-discrimination training and testing*

Studies were conducted in operant-conditioning chambers (model ENV-008; MED Associates, St. Albans, VT) that were individually enclosed in larger lightproof Malaguard sound-attenuating cubicles (model ENV-022M; MED Associates) and modified to accommodate murine subjects. The side wall of each chamber compartment used in these studies was equipped with a spout

through which liquid reinforcement was delivered, driven by an infusion pump mounted outside the chamber but within the cubicle. The spout was centered between two retractable levers and positioned just beneath a red stimulus light, which was illuminated during reinforcer delivery. Mice were trained 5 days per week under a fixed-ratio (FR) schedule of reinforcement wherein completion of the response requirement on either lever was reinforced by 2 s of access to a palatable liquid reinforcer (approximately 0.02 ml of vanilla-flavored coffee creamer diluted 1:1 with water) followed by a 10-s timeout (TO) before programmed consequences were reinitiated. Once a response requirement was met on either lever, that lever was retracted and subjects were required to meet the response requirement on the other lever. When the response requirement was met on each of the levers, both levers were reintroduced after the TO. In this manner, mice received equivalent reinforcement from each lever, and no subsequent biases for one lever or the other were noted. Animals acquired lever-pressing behavior on a FR1 schedule of reinforcement in sessions lasting 60 min or until 60 reinforcers had been earned, whichever came first. The FR value increased by one for every 20th reinforcer earned within a given session, and the FR value achieved was carried over between sessions until mice were responding under an FR10. This segment of the training was complete when mice performed stably over five consecutive FR10 sessions.

Next, each group of mice was trained in 30-min sessions 5 days per week to discriminate their respective drug [1.5 mg/kg *S*(+)-MDMA or *R*(−)-MDMA administered intraperitoneally] from saline vehicle. Injections were administered

10 min before extension of the response levers, signaling the start of the behavioral session. During discrimination training, a single response on the injection-inappropriate lever resulted in retraction of that lever and extinction of the house light for a 30-s TO. During this TO, the injection-appropriate lever remained extended into the chamber, but responses on it had no programmed consequences. After the elapse of the TO, completion of the ratio on the remaining, injection-appropriate lever was reinforced. Percentage of drug-appropriate responding was calculated as the number of reinforcers earned divided by the total number of opportunities to make a choice between the two levers, multiplied by 100. Training was composed of an alternating schedule of drug or saline injection. Subjects were switched from saline to drug or vice versa for the next day of training if they achieved a criterion of greater than 80% correct choices or after three consecutive training days where performance was below criterion. In the latter case, a single day of FR10 responding (as in the lever training condition) was imposed to reestablish contact with the reinforcement contingencies and to increase behavioral output before discrimination training was resumed.

Drug-induced stimulus control was assumed to be present when, in five consecutive sessions, animals made 80% or more correct choices. After stimulus control was established with the training drugs, tests were conducted once per week in each animal as long as performance did not fall below the criterion level of 80% correct responding in any one of the previous three training sessions. Approximately half of the test sessions were conducted the day after saline

training sessions with the remainder following drug training sessions. During test sessions, a multiple component cumulative dosing procedure was used, and no responses were reinforced. Each component was terminated after the emission of 10 responses on either lever. Mice were then removed from the chamber, administered the next cumulative dose, and returned to the chamber. Ten minutes later, levers were re-extended into the experimental space. In this manner, four doses of drug could be tested in a single session, over approximately 40 min. The distribution of responses between the two levers was expressed as a percentage of total responses emitted on the drug-appropriate lever. Response rate was calculated for each session by dividing the total number of responses emitted on both levers by the elapsed time before 10 responses on either lever.

Complete generalization of a training drug to a test drug was determined when 1) a mean of 80% or more of all test responses occurred on the drug-appropriate lever and 2) there was a statistically significant difference between the response distributions of the test drug and saline control sessions. An intermediate degree of generalization was defined as being present when response distributions after a test drug were less than 80% drug-appropriate and were significantly different from saline control sessions. Finally, when the response distribution after a test drug was not statistically significantly different from that in saline control sessions, an absence of generalization of the training drug to the test drug was assumed. Failure to complete an FR10 on either lever

within 2 min terminated the sessions and indicated disruption of schedule-controlled behavior.

*Sleep studies*

In order to quantify sleep-wake patterns, female subjects were outfitted with Actiwatch (Mini Mitter, Bend, OR, USA) activity monitors. The Actiwatch consists of an omni-directional sensor that is sensitive to motion (recorded as activity counts) and has been previously shown to be a reliable, non-invasive method for sleep monitoring (Sadeh et al., 1995; Kushida et al., 2001). The monitors were programmed to record the total piezo-electric voltage generated over the preceding 60 s (i.e. epoch length=60 s). The devices record intensity, amount and duration of movement in all three planes by producing a voltage that is subsequently converted to an arbitrary count and data logged (for review see Mann et al. 2005). The Actiwatch sensor was initially attached to a given subject's collar while the subject was under ketamine (3.0-10mg/kg, i.m.) anesthesia. Sleep measurements for the subsequent 48 hours were not included in the analysis in order to allow the subject to recover completely from anesthesia. Sleep measurements were obtained each night from all subjects until the data capacity of the monitor was reached (approximately every 45 days). Then, a new monitor was attached to the subject's collar while the subject was anesthetized and 48 hours later sleep monitoring was continued. Subjects were administered an i.m. injection of a test compound or drug vehicle each Monday and Wednesday at 17:30 and 18:30 (30 minutes prior to the beginning of the

dark period) while the subject was in its colony home cage. The order of the doses and drugs administered was randomized across subjects. The data were downloaded and analyzed with Actiware Sleep 3.4 (Mini-Mitter Co. Inc., Bend, OR, USA). Sleep measurements were automatically calculated from the underlying activity counts using a temporal smoothing algorithm on the basis that sleeping or wakefulness are generally continuous behaviors. The following sleep parameters were assessed: sleep latency (time elapsed between the beginning of the dark period and the first period the subject was judged to be asleep) and sleep duration (total time the subject was judged to be asleep over the entire 12 hour dark period). Initial positive control experiments were undertaken to establish the effects of the known psychomotor-stimulant amphetamine and to the determine the appropriate conditions for administration of the selective 5-$HT_{2A}$ receptor antagonist M100907 (Pehek et al., 2006). The dose of M100907 (0.3 mg/kg) was chosen on the basis that it had been previously shown to antagonize self-administration of MDMA in rhesus monkeys (Fantegrossi et al., 2002). Finally, drug interactions studies were undertaken with each form of MDMA to determine the role of the 5-$HT_{2A}$ receptor in any sleep-disrupting effects that they elicited.

*Data Analysis*

Graphical presentation of all data depicts mean ± S.E.M. All graphical data presentations were created by using GraphPad Prism 4 (GraphPad Software

Inc., San Diego, CA), all statistical tests were performed by using SigmaStat 3 (Systat Software, Inc., San Jose, CA), and significance was judged at $P < 0.05$.

*Discrimination data analysis*

Drug-discrimination data are expressed as percentage of drug-appropriate responding, which is the number of responses emitted on the drug-appropriate lever as a percentage of the total number of responses emitted. Response rates are expressed as the number of responses per second, calculated for each session by dividing the total number of responses emitted (before the emission of 10 responses on either lever) by elapsed time. Data for any subjects failing to emit 10 responses within 2 min of lever extension were deemed to be behaviorally disrupted and were not considered in the calculation of the percentage of drug-appropriate responding or response rates. Generalization was said to occur if 80% or more of the responses were on the drug-appropriate lever. The statistical significance of the generalization of a training drug was determined using one-way repeated measure analysis of variance (ANOVA) to compare the two training conditions with the test drug. Subsequent multiple comparisons to saline control were made by the method of Dunnett. Control data were repeated for each comparison, and statistical analyses were applied by using the appropriate control sessions. However, for purposes of clarity, mean values for control data are shown in all figures. Nonlinear regression analysis with a variable slope sigmoidal dose-response curve was used to calculate the dose that was 50% effective ($ED_{50}$; with a set range of 0–100%) and the Hill

slope of the dose-effect curve when the test compound partially or fully substituted for the training drug. The equation used for this analysis was Y = 0 + (100/1+10((LogED50 – X) * Hill Slope)), where X is equal to the logarithm of the dose and Y is equal to the response.

*Sleep data analysis*

The primary dependent variables tested in this experiment were the latency from the time the colony lights were turned off to the first sleep bout and the total duration of sleep over the 12 hour dark epoch. Amphetamine data were analyzed via a one-way repeated measures (RM) analysis of variance (ANOVA) with correction for multiple comparisons utilizing Dunnett's method versus vehicle treatment. Data were analyzed for each form of MDMA via a one-way RM ANOVA with post-hoc correction by Dunnett's method compared to saline treatment. Furthermore, M100907 data across different pretreatment times were analyzed via one-way RM ANOVA with correction for multiple comparisons utilizing Dunnett's method versus amphetamine treatment. Data were analyzed for each form of MDMA subsequent to treatment with either vehicle or the selective 5-$HT_{2A}$ receptor antagonist M100907 via a two-way RM ANOVA with dose and pretreatment as the main factors. Individual comparisons were then drawn across dose via a one-way RM ANOVA with correction for multiple comparisons utilizing Dunnett's method versus vehicle treatment. Differences at each dose due to treatment with either vehicle or M100907 were assessed via a paired t-test.

## Results

### Discrimination results

Under the procedures used, all animals learned to reliably discriminate the training dose of the respective training drug from saline. Once subjects were fully trained, group means of each group for drug-appropriate lever responding were greater than 90% subsequent to administration of the training dose and less than 10% subsequent to administration of saline. One-way repeated measures ANOVA was carried out for all drug substitutions in each respective group. A significant main effect of condition (drug and dose) was found for both the *S*(+)-MDMA ($F_{5,17}$ = 12.706; $P < 0.001$) and the *R*(−)-MDMA groups ($F_{5,18}$ = 7.141; $P < 0.001$).

#### *Amphetamine Substitution (Figure 2-2)*

Cumulative administration of *S*(+)-amphetamine engendered dose-dependent and full substitution (100 ± 0%) in *S*(+)-MDMA-trained subjects. However, *S*(+)-amphetamine did not substitute for the *R*(−)-MDMA cue up to doses that suppressed responding. Post hoc analysis by means of the Dunnett's test revealed that the 1.0 and 3.0 mg/kg doses of *S*(+)-amphetamine were significantly different from saline administration ($P < 0.05$) in the *S*(+)-MDMA-trained animals. No dose of amphetamine was significantly different from saline in the *R*(−)-MDMA-trained subjects. Nonlinear curve fitting determined an $ED_{50}$ of 0.42 mg/kg with a Hill slope of 2.48 ($R^2$ = 0.79) in the *S*(+)-MDMA-trained

animals. This analysis was not possible in *R*(−)-MDMA-trained mice due to the failure of amphetamine to substitute in these subjects.

*Cocaine Substitution (Figure 2-3)*

Cumulative administration of cocaine engendered dose-dependent and full substitution (100 ± 0%) in *S*(+)-MDMA-trained subjects. In the *R*(−)-MDMA-trained subjects, cocaine dose-dependently and partially substituted (66.67 ± 33%) for the training dose. Post hoc analysis by means of the Dunnett's test revealed that the 1.0 and 3.0 mg/kg doses of cocaine were significantly different from saline administration ($P < 0.05$) in the *S*(+)-MDMA-trained animals, and that 3.0 mg/kg cocaine was significantly different from saline ($P < 0.05$) in the *R*(−)-MDMA-trained animals. Nonlinear curve fitting determined an $ED_{50}$ of 0.36 mg/kg with a Hill slope of 1.63 ($R^2 = 0.60$) in the *S*(+)-MDMA-trained animals. Cocaine was approximately five times less potent in the *R*(−)-MDMA-trained animals with an $ED_{50}$ of 1.54 mg/kg and a Hill slope of 0.95 ($R^2 = 0.60$).

*2C-T-7 Substitution (Figure 2-4)*

Cumulative administration of 2C-T-7 engendered dose-dependent and full substitution (84.25 ± 11.76%) in *R*(−)-MDMA-trained subjects; however, 2C-T-7 did not substitute for the *S*(+)-MDMA cue (20.0 ± 20.0%). Post hoc analysis by means of the Dunnett's test revealed that the 0.3, 1.0, and 3.0 mg/kg doses of 2C-T-7 were significantly different from saline administration ($P < 0.05$) in the *R*(−)-MDMA-trained animals. No dose of 2C-T-7 was significantly different from

saline in the *S*(+)-MDMA-trained subjects. Nonlinear regression analysis could not accurately fit the data for 2C-T-7 in the *R*(−)-MDMA-trained animals. This result was probably due to the lack of multiple intermediate substitution data points at the doses used. Likewise, the failure of 2C-T-7 to substitute for the *S*(+)-MDMA training dose precluded this level of analysis in these subjects as well.

*DPT Substitution (Figure 2-5)*

Cumulative administration of DPT engendered dose-dependent and full substitution in subjects trained with *R*(−)-MDMA (100 ± 0%) and in subjects trained with *S*(+)-MDMA (96.15 ± 3.847). Post hoc analysis by means of the Dunnett's test revealed that the 1.0 and 3.0 mg/kg doses of cocaine were significantly different from saline administration ($P < 0.05$) in the *R*(−)-MDMA-trained animals, whereas the 1.0, 3.0, and 10.0 mg/kg doses of DPT were significantly different from saline ($P < 0.05$) in the *S*(+)-MDMA-trained animals. Nonlinear curve fitting determined an $ED_{50}$ of 0.14 mg/kg with a Hill slope of 1.09 ($R^2 = 0.58$) for DPT in the *R*(−)-MDMA-trained animals. DPT was approximately six times less potent in the *S*(+)-MDMA-trained animals with an $ED_{50}$ of 0.91 mg/kg and a Hill slope of 2.2 ($R^2 = 0.75$).

Sleep results

*Effects of amphetamine (Figure 2-6)*

Initial experiments examined the effects of amphetamine, as a positive control, to establish the effects of a known psychomotor-stimulant on sleep

behavior in rhesus monkeys, under the conditions employed. A one-way RM ANOVA revealed a significant main effect of amphetamine on both latency to ($F_{4,3}$ = 15.280; $p < 0.001$) and duration of ($F_{4,3}$ = 138.587; $p < 0.001$) sleep. The powers of these tests were 1.000 and 0.999, respectively.

*Effects of MDMA (Figure 2-7)*

The effects of each form of MDMA on sleep were then examined. One-way RM ANOVA showed a significant main effect of S,R(+/-)-MDMA ($F_{4,3}$ = 8.234; $p = 0.003$) and S(+)-MDMA ($F_{4,3}$ = 8.312; $p = 0.003$) on sleep latency but no effect of R(-)-MDMA ($F_{4,3}$ = 2.255; $p = 0.134$; Figure 2-6, A). The powers of these tests were 0.931, 0.934 and 0.249, respectively. Post-hoc testing, via Dunnett's method, showed that treatments with S,R(+/-)-MDMA were significantly different from saline at 1.0, 1.7, and 3.0 mg/kg and treatments with S(+)-MDMA were significantly different at 1.0 and 1.7 mg/kg. For sleep duration, one-way RM ANOVA showed a significant main effect of S(+)-MDMA ($F_{4,3}$ = 15.068; $p < 0.001$) but no main effect S,R(+/-)-MDMA ($F_{4,3}$ = 2.269; $p = 0.133$) or R(-)-MDMA ($F_{4,3}$ = 0/057; $p = 0.981$; Figure 2-6, B). The powers of these tests were 0.999, 0.252 and 0.050, respectively. Post-hoc testing, via Dunnett's method, showed that treatments with S(+)-MDMA were significantly different at 1.0 and 1.7 mg/kg.

*Influence of pretreatment time on the effects of M100907 (Figure 2-8)*

Next, as a further positive control experiment, the effects of 5-$HT_{2A}$ receptor antagonism on sleep disruption by amphetamine were determined via pretreatments with the selective antagonist M100907. The dose of M100907 (0.3

mg/kg) was chosen on the basis that it had been previously shown to antagonize self-administration of MDMA in rhesus monkeys (Fantegrossi et al., 2002). However, initial experiments showed that M100 was marginally effective at antagonizing the effects of amphetamine on sleep. Therefore, the pretreatment time of M100 was systematically increased to determine if there are conditions under which it is more effective. A one-way RM ANOVA revealed a significant main effect of pretreatment time on the effectiveness of M100 to antagonize amphetamine's effects on both latency to ($F_{4,3}$ = 16.358; $p < 0.001$) and duration of ($F_{4,3}$ = 15.514; $p < 0.001$) sleep. The powers of these tests were 0.999 and 0.999, respectively.

*Effects of M100907 on S,R(+/-)-MDMA-elicited sleep disruption (Figure 2-9)*

The effects of 5-$HT_{2A}$ receptor antagonism on sleep disruption by each form of MDMA were then evaluated via pretreatments with M100907. M100907 was administered at the pretreatment time (60 mins) found to most effectively attenuate the effects of amphetamine. For the effects of S,R(+/-)-MDMA, following pretreatment with M100907 or its vehicle, two-way RM ANOVA revealed a significant main effect of dose ($F_{4,3}$ = 7.108; $p = 0.005$) and pretreatment ($F_{4,1}$ = 32.947; $p = 0.005$) and a significant interaction ($F_3$ = 3.909; $p = 0.037$) for sleep latency (Figure 2-9, A) and a significant main effect of pretreatment ($F_{4,1}$ = 75.611; $p < 0.001$) but not dose ($F_{4,3}$ = 1.164; $p = 0.364$) and no significant interaction ($F_3$ = 2.267; $p <0.001$) for sleep duration (Figure 2-9, B). The powers of these tests were 0.985, 0.880, 0.539, 1.000, 0.071, and 0.251, respectively. Post-hoc analysis via one-way RM ANOVA revealed no effect of

S,R(+/-)-MDMA following pretreatment with M100907 on sleep latency ($F_{4,3}$ = 0.358; p = 0.784). The power of this test was 0.050. Post-hoc analysis via a paired t-test revealed that following pretreatment with either M100907 or its vehicle the effects of S,R(+/-)-MDMA on sleep latency were significantly different at 1.0 ($t_4$ = 5.410; p = 0.006), 1.7 ($t_4$ = 2.424; p = 0.042), and 3.0 mg/kg ($t_4$ = 4.035; p = 0.016). Furthermore, M100907 had no effects on this measure in its own right as sleep latency was not significantly different following administration of saline subsequent to pretreatment with M100907 or its vehicle ($t_4$ = -0.283; p = 0.791). The powers of these tests were 0.975, 0.390, 0.833, and 0.050, respectively. For sleep duration, the effects of S,R(+/-)-MDMA on sleep latency were significantly different at 3.0 ($t_4$ = -3.873; p = 0.018) but not 1.0 ($t_4$ = -0.517; p = 0.633) or 1.7 mg/kg ($t_4$ = -1.913; p = 0.128). Again, M100907 had no effect on this measure in its own right as sleep latency was not significantly different following administration of saline subsequent to pretreatment with M100907 or its vehicle ($t_4$ = -0.973; p = 0.385). The powers of these tests were 0.802, 0.050, 0.238, and 0.050, respectively. The effects of S,R(+/-)-MDMA are presented for illustrative purposes but were not included in any of these analyses.

*Effects of M100907 on S(+)-MDMA-elicited sleep disruption (Figure 2-10)*

In addition, the effects of 5-$HT_{2A}$ receptor antagonism on the sleep disruption by S(+)-MDMA was evaluated. Following pretreatment with M100907 or its vehicle, two-way RM ANOVA revealed that S(+)-MDMA engendered a significant main effect of dose ($F_{4,3}$ = 15.443; p < 0.001) but not pretreatment ($F_{4,1}$ = 1.489; p = 0.289) for sleep latency (Figure 2-10, A) and a significant main

effect of both dose ($F_{4,3}$ = 9.192; p = 0.002) and pretreatment ($F_{4,1}$ = 36.087; p = 0.004) for sleep duration (Figure 2-10, B). The powers of these tests were 0.999, 0.084, 0.959, and 0.991, respectively. The significance of the interaction between these factors could not be calculated for S(+)-MDMA as not all doses of S(+)-MDMA were administered subsequent to M100907. Post-hoc analysis via one-way RM ANOVA revealed an effect of S(+)-MDMA following pretreatment with M100907 on sleep latency ($F_{4,2}$ = 12.341; p = 0.004) and, using Dunnett's method, that S(+)-MDMA was significantly different from saline at 1.7 mg/kg. The power of this test was 0.949. For sleep duration, post-hoc analysis one-way RM ANOVA revealed no effect of S(+)-MDMA following pretreatment with M100907 ($F_{4,2}$ = 0.534; p = 0.606). Furthermore, post-hoc analysis via a paired t-test revealed that following pretreatment with either M100907 or its vehicle the effects of S(+)-MDMA on sleep duration were not significantly different at 1.0 ($t_4$ = -2.467; p = 0.069) or 1.7 mg/kg ($t_4$ = -2.288; p = 0.084). The powers of these tests were 0.403, and 0.348, respectively.

*Effects of M100907 on R(-)-MDMA-elicited sleep disruption (Figure 2-11)*

Finally, the effects of 5-$HT_{2A}$ receptor antagonism on the sleep disruption by R(-)-MDMA was evaluated. The results of these drug interaction experiments for sleep latency and sleep duration are presented for illustrative purposes in Figure 2-11 A and B, respectively. However, no statistical analysis was carried out on the M100907 pretreament data as R(-)-MDMA did not significantly affect sleep following pretreatment with vehicle

## Discussion

The aim of the present study was to supplement and extend previous studies of the behavioral effects of MDMA and its stereoisomers. In the first set of experiments, the nature of the interoceptive cue engendered by *S*(+)- and *R*(−)-MDMA was examined, under conditions that parametrically varied both the chemical similarity of the test drugs to MDMA and the pharmacological selectivity of the test compounds. It is important to note that in the case of both the stimulants (amphetamine and cocaine) and hallucinogens (2C-T-7 and DPT) tested, the pharmacological effects of the phenethylamine-based drugs (amphetamine and 2C-T-7) were more selective than were the effects of the drugs not structurally related to MDMA (cocaine and DPT). Under the procedures used, all animals learned to reliably discriminate the training dose of *S*(+)-MDMA or *R*(−)-MDMA from saline. In combination with previous reports comparing the interoceptive effects of each isomer of MDMA to *N*-substituted piperazines (Yarosh et al., 2007) or to each other (Fantegrossi et al., 2009), these data indicate that mice can be reliably trained to discriminate each isomer of MDMA from saline.

*S*(+)-amphetamine is structurally similar to MDMA, and both compounds stimulate substrate-based release of monoamines (Acquas et al., 2007; Fleckenstein et al., 2007). Based on previous reports describing the neurochemical effects of these compounds, and based on the discriminative profiles of the MDMA isomers established in rats, we hypothesized that the

interoceptive effects of amphetamine should be more similar to those of *S*(+)-MDMA than those of *R*(−)-MDMA in the mouse. This hypothesis was confirmed by the full substitution of *S*(+)-amphetamine for the discriminative cue of *S*(+)-MDMA and the failure of amphetamine to engender significant *R*(−)-MDMA-like responding in mice. 2C-T-7 is also structurally similar to MDMA, and both compounds are substituted phenethylamines with agonist affinity for the 5-$HT_{2A}$ receptor (Lyon et al., 1986; Fantegrossi et al., 2005, 2008). Based upon this pharmacological profile and previous work on the interoceptive effects of the MDMA enantiomers in rats, we hypothesized that the interoceptive effects of 2C-T-7 should be more similar to those of *R*(−)-MDMA than to those of *S*(+)-MDMA in mice. This hypothesis was also confirmed by the full substitution of 2C-T-7 in the *R*(−)-MDMA-trained animals and by the lack of *S*(+)-MDMA-like responding elicited by 2C-T-7 in *S*(+)-MDMA-trained animals. The sum of these two data sets indicates a profound qualitative difference in the discriminative cue engendered by each stereoisomer of MDMA. These data support previous studies that contrast the stimulus properties of the two isomers in rats (Glennon et al., 1988; Baker et al., 1995). Furthermore, the distinct interoceptive effects of the two isomers of MDMA have now been demonstrated across different operant schedules, training procedures, training doses, training drugs (generalization versus substitution), and species. However, it remains difficult to explain the capacity of each MDMA enantiomer to substitute for one another in mice, using procedures identical to those herein described previously (Fantegrossi et al., 2009). Nevertheless, these present findings suggesting qualitatively distinct

interoceptive effects in the mouse are supported not only by earlier drug-discrimination experiments in the rat, but also by previous studies where *S*(+)-MDMA-elicited stimulant-like effects, while *R*(−)-MDMA induced hallucinogen-like effects, on multiple behavioral and physiological endpoints in mice (Fantegrossi et al., 2003, 2005).

It is also clear from the present studies that, when the structural and pharmacological similarity of the test compounds to MDMA was reduced, the previously observed qualitative differences between the interoceptive effects of the MDMA enantiomers were replaced by simple potency differences. Cocaine has no notable structural features in common with MDMA, and although both compounds alter synaptic monoamine levels, MDMA does so through substrate based release (Setola et al., 2003) while cocaine passively blocks monoamine reuptake (Kuhar et al., 1999). Nevertheless, cocaine fully substituted for the training dose of *S*(+)-MDMA and partially substituted for the interoceptive cue induced by *R*(−)-MDMA. In mice trained with *R*(−)-MDMA, the Hill slope of the cocaine dose-effect curve was 1.63, and only three of the six animals reached the response criterion at the highest dose of cocaine tested, suggesting that full substitution might have been achieved were it not for the rate suppressant effects of cocaine under these conditions in these subjects. Although it is not clear why the rate-suppressant effects of cocaine were more pronounced in mice trained with *R*(−)-MDMA than in mice trained with *S*(+)-MDMA, it is important to note that the interoceptive effects of cocaine were approximately five times more potent in the *S*(+)-MDMA group than in the *R*(−)-MDMA-trained animals. Thus, although

cocaine substituted for each MDMA isomer, a conspicuous potency difference remained. Likewise, the chemical structures of MDMA and DPT are not particularly congruent. In addition to its agonist affinity for 5-$HT_{2A}$ receptors, DPT functionally inhibits reuptake of monoamines without stimulating release (Nagai et al., 2007). DPT fully substituted for the training dose of each stereoisomer but was 6-fold more potent in mice trained with *R*(−)-MDMA. These data are in general agreement with previous studies showing that LSD and cocaine, drugs with promiscuous pharmacological profiles that are structurally dissimilar to MDMA, partially or fully substituted for each isomer (Baker at al., 1995 and Bondareva et al., 2005, respectively). Taken together, these data reveal that of the compounds tested in this study, *R*(−)-MDMA shares stimulus properties with direct 5-$HT_{2A}$ receptor agonists and indirect serotonin agonists, whereas *S*(+)-MDMA shares stimulus properties with only indirect agonists in mice. Furthermore, *S*(+)-MDMA shares stimulus properties with selective dopamine substrate releasers and nonselective monoamine reuptake inhibitors, whereas *R*(−)-MDMA shares stimulus properties with only nonselective monoamine reuptake inhibitors.

It is important to note that many procedural variables can profoundly affect the results of drug-discrimination studies. In particular, the training dose chosen to establish discriminative control affects both the rate of acquisition of the discrimination task and the “sensitivity” of the animals to subsequent test compounds. For example, rats trained to discriminate 40 mg/kg of the μ-opioid agonist fentanyl from saline acquired the discrimination more rapidly than did

animals trained with lower doses, but the dose-effect functions for fentanyl discrimination in these animals were shifted to the right compared with rats trained with lower fentanyl doses (Colpaert et al., 1980). The role of various procedural variables, including training dose, in drug-discrimination experiments involving serotonergic compounds has been reviewed previously (Winter et al., 1999). Thus, whereas the presently reported data are in accordance with previous experiments conducted in rats (Glennon et al., 1988; Baker et al., 1995), further drug-discrimination experiments with the MDMA enantiomers in mice trained with both higher and lower doses are warranted.

In summary, these data indicate that the discriminative cues mediated by each enantiomer of MDMA are distinct, yet overlapping, and further suggest that, as has been demonstrated in the rat, the interoceptive effects of *S*(+)-MDMA are primarily stimulant-like, whereas those of *R*(−)-MDMA are predominantly hallucinogen-like in the mouse. The stimulus properties of amphetamine, a relatively dopamine-selective stimulant, or 2C-T-7, a relatively selective serotonergic hallucinogen, completely dissociate the MDMA enantiomers from one another. In contrast, the interoceptive effects of cocaine, a stimulant with approximately equivalent effects on dopamine, norepinephrine, and serotonin, or DPT, a hallucinogen that also inhibits reuptake of serotonin, generalize to both MDMA enantiomers, but do so more potently in the more stimulant-like *S*(+)-MDMA or the more hallucinogen-like *R*(−)-MDMA, respectively. This pattern of findings would seem to suggest that the interoceptive effects of the MDMA enantiomers in mice are mediated by a mixture of dopaminergic and serotonergic

components. In the case of *S*(+)-MDMA, the dopaminergic component is a more salient cue than the serotonergic component, whereas the reverse is true for *R*(−)-MDMA. This notion may explain not only the present results, but also the capacity for each enantiomer to substitute for one another in mice (Fantegrossi et al., 2009).

In the second set of experiments, the effects of each form of MDMA on sleep in rhesus monkeys were examined. The primary impetus for these experiments was the lack of cohesive data in regards to the stimulant-like effects of MDMA and in particular its effects in nonhuman primates. Although MDMA has been previously shown to reliably function as a robust psychomotor-stimulant in rodents and humans the results of studies utilizing nonhuman primates have been more ambiguous. For example, MDMA is reliably self-administered by nonhuman primates (Beardsley et al., 1986; Lamb and Giffiths 1987; Fantegrossi et al., 2002; Fantegrossi et al., 2004; Lile et al., 2005; Wang and Woolverton 2007; Banks et al., 2008) but does not appear to function as a behavioral (Fantegrossi et al. 2009) or a locomotor-stimulant (Taffe et al., 2006) in this taxa. MDMA is often abused at all night “rave” parties and has been previously shown to disrupt sleep in humans (Green et al., 2003; Randall et al., 2009). Therefore, we suggest that any stimulant-like effects of MDMA may be more likely to be expressed in the context of sleep disruption than other metrics consistent with stimulant-like effects. As such, the goals of these experiments were to: 1) determine the effects of MDMA on sleep in rhesus monkeys, 2) determine if the

stereoisomers of MDMA produce differential effects on sleep, and 3) determine if the effects of any form of MDMA on sleep could be effectively antagonized.

Relatively few determinations of acute drug effects on sleep have been carried out in rhesus monkeys. Therefore, initial experiments were carried out with amphetamine, in order to validate the procedures used. As expected, administration of amphetamine dose-dependently increased sleep latency and decreased sleep duration. Further, experiments were undertaken to validate the effects of 5-$HT_{2A}$ receptor antagonism on the sleep-disrupting effects of amphetamine. These experiments were carried out, in part, as a positive control for the effects of this receptor on MDMA-elicited sleep disruption. However, 5-$HT_{2A}$ receptor antagonism of amphetamine elicited sleep disruption was also evaluated due to reports suggesting that it may be viable pharmacotherapeutic for stimulants other than MDMA (Gudelsky et al., 1994; McMahon and Cunningham, 2001; Bubar and Cunningham, 2006). Despite the promise of these studies, other work suggests that pharmacotherapeutic potential of the 5-$HT_{2A}$ receptor may be limited to MDMA as selective antagonism of this receptor by M100907 attenuated MDMA but not cocaine self-administration (Fantegrossi et al., 2002). In the present study, administration of M100907 at the same dose and pretreatment time used by Fantegrossi and colleagues (2002) produced no marked diminution of the sleep-disrupting effects of amphetamine. Further study showed, however, that as the pretreatment of M100907 was increased it became more effective. In fact, M100907 significantly decreased sleep disruption by amphetamine at both a 30 and 60 minute pretreatment time. This provides strong

support for the continued evaluation of the role of this receptor in the behavioral effects of psychomotor-stimulants and refined the subsequent examination of its role in the sleep-disrupting effects of MDMA.

Substantial evidence suggests that abuse of MDMA disrupts normal sleep patterns (for a review see (Schierenbeck et al., 2008). However, the acute effects of MDMA on sleep have been rarely studied. Nevertheless, the few studies to do so indicate a capacity to disrupt sleep. For example, Gouzoulis and colleagues (1992) found that the MDMA derivative methylenedioxyethylamphetamine (MDE) eliminated REM sleep in human subjects. Further study showed that MDMA increased vigilance and psychomotor function when subjects were required to stay awake overnight (Kuypers et al., 2007). Most recently, MDMA has been shown to directly increase sleep latency, also in human subjects (Randall et al., 2009). Consistent with these effects, in this study, racemic MDMA disrupted sleep latency but not sleep duration in rhesus monkeys. MDMA was administered at doses that are equivalent, on a mg/kg basis, to the doses of MDMA typically abused by humans (Cole et al., 2002; Harris et al., 2002; Green et al., 2003) and are within the range that rhesus monkeys will voluntarily self-administer (Fantegrossi et al., 2002; Banks et al., 2008). Furthermore, these doses are similar to those used in the studies of the acute effects of MDMA on sleep in humans. In those studies, MDMA was administered at approximately 1 to 2 mg/kg. In the present report, MDMA dosing continued up to 3.0 mg/kg without any significant disruption of sleep duration. However, it is possible racemic MDMA would disrupt sleep duration at higher doses. Nevertheless, the

effects of MDMA on sleep latency and its concordance to clinical studies clearly demonstrates the capacity of MDMA to disrupt sleep. Thus, this work reveals new conditions under which MDMA can function as a psychomotor-stimulant in nonhuman primates.

An important caveat to the results of these studies is that they were generated using non-invasive actigraphic methods rather then the polysomnographic methods traditionally used in sleep studies. However, it is important to note that this actigraphic approach is a valid proxy for polysomnogrphic techniques as the two approaches exhibit greater than 90% agreement (Sadeh et al., 1995; Kushida et al., 2001). Nevertheless, small motor movements that do not involve the entire body, such as isolated head or limb movements, be not be accurately recorded by actigraphy (Papailiu et al., 2007). As such, it is possible that actigraphy may overcount sleep duration (Barrett et al., 2009). Despite this limitation, the presently reported data are highly concordant with those obtained, using polysomnographic techniques, in human subjects following acute administration of MDMA. (Randall et al., 2009).

These findings were extended by determining the effects of the stereoisomers of MDMA on sleep. S(+)-MDMA has been shown to more readily function as a psychomotor-stimulant than R(-)-MDMA (Glennon et al., 1988; Baker et al., 1995; Murnane et al., 2009). As such, we predicted that S(+)-MDMA would more effectively disrupt sleep than R(-)-MDMA. The results of this study confirm this prediction. Furthermore, unlike racemic MDMA, S(+)-MDMA

decreased the total duration of sleep over the 12 hour epoch of darkness. This suggests S(+)-MDMA may be an even more effective psychomotor-stimulant than S,R(+/-)-MDMA. However, differential effects on duration of sleep could be due to a different duration of action between these forms of MDMA. An *in vivo* study of the time-course of the neurochemical effects of each form of MDMA could be particularly informative in this regard. Perhaps most importantly, R(-)-MDMA had no significant effect on either sleep latency or sleep duration. These data show clear concordance with the previously described discrimination data. In combination, these data sets show a strong dissociation of the intereoceptive and behavioral effects of each stereoisomer of MDMA, across both species and assay.

These results were further extended by determining if selective antagonism of the 5-$HT_{2A}$ receptor by M100907 would attenuate the effects of MDMA on sleep. Currently, no pharmactherpies are available for MDMA abuse. It is possible that a drug that attenuates the behavioral effects of MDMA in preclinical studies may be of clinical utility in the treatment of MDMA abuse. As such, the 5-$HT_{2A}$ was targeted because antagonism of this receptor, using M100907, has been previously shown to attenuate MDMA self-administration in rhesus monkeys (Fantegrossi et al., 2002). Furthermore, in this study, initial determinations of its effects on sleep disruption by amphetamine were positive. Consistent with these findings, selective antagonism of the 5-$HT_{2A}$ receptor completely eliminated the sleep-disrupting effects of racemic MDMA. The interaction between 5-$HT_{2A}$ antagonism and the effects of S(+)-MDMA were more

complex. When pretreated with vehicle, S(+)-MDMA significantly disrupted both sleep onset and sleep duration. When pretreated with M100907, S(+)-MDMA had no significant effect on sleep duration and only disrupted sleep onset at the highest dose administered. However, post-hoc comparisons at each dose of S(+)-MDMA between vehicle and M100907 pretreatment did not reach statistical significance. This may be, in part, due to the nature of interaction between 5-$HT_{2A}$ antagonism and the effects of S(+)-MDMA. Visual inspection of the data suggests that 5-$HT_{2A}$ may be decreasing the potency of S(+)-MDMA rather than eliminating its effects, an effect that is indicated by a “right-ward” shift in a dose effect curve. This would be consistent with the effects of 5-$HT_{2A}$ antagonism on self-administration of each form of MDMA (Fantegrossi et al., 2002), as a similar pattern of effects emerged in that study. However, this may also be an artifact of the variance in this data set. A new study with an expanded sample size may be necessary to statistically capture any differences in the effects of 5-$HT_{2A}$ antagonism on sleep disruption by each form of MDMA. Nevertheless, these results support the continued study of the 5-$HT_{2A}$ receptor as a novel pharmacotherapeutic target for treating MDMA abuse. A study that determines whether antagonism of this receptor alters the neurochemical effects of MDMA may be of particular value in the continued development of this receptor as a pharmacotherapeutic target.

In conclusion, these studies further demonstrate that MDMA elicits effects consistent with both psychomotor-stimulants and hallucinogens. Furthermore, these effects segregate coherently across its stereoisomers. In addition, this

work supports continued study of the 5-$HT_{2A}$ receptor as a viable pharmacotherapeutic target for treating MDMA abuse. Finally, in so far as preclinical experiments can be generalized to human subjects, it seems likely that isomers possessing distinct but overlapping interoceptive effects could produce a racemic mixture with complex subjective effects, as is the case with MDMA. Further research into the intriguing subjective effects of MDMA, particularly as they relate to underlying neuropharmacological effects of the racemic mixture and its stereoisomers, would be informative.

**Figure 2-1.** Chemical structures of all substitution compounds tested in MDMA discrimination experiments.

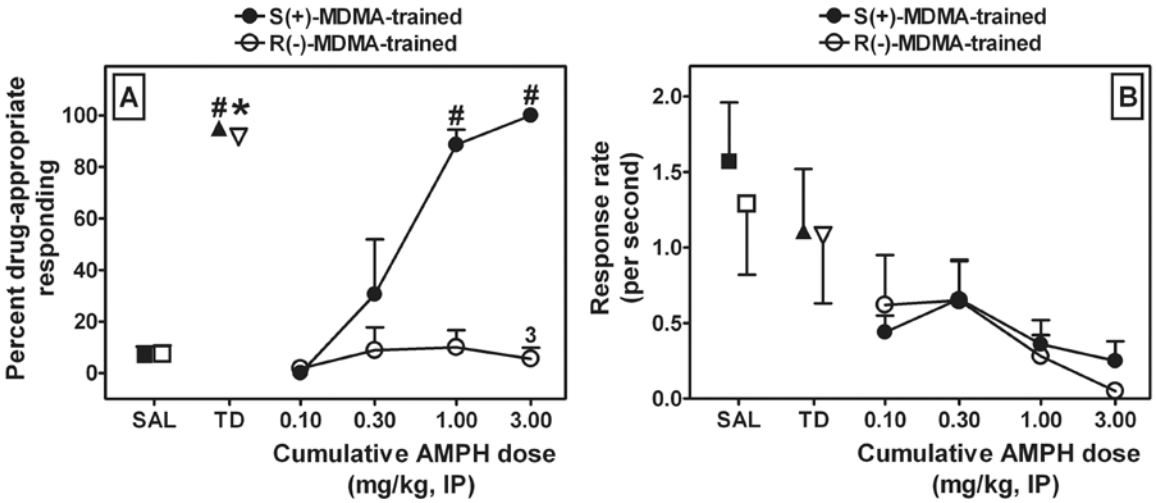


**Figure 2-2.** Effects of amphetamine in mice trained to discriminate 1.5 mg/kg S(+)-MDMA (closed circles) or 1.5 mg/kg R(-)-MDMA (open circles) from saline (N=6 per group). All points represent the mean ± SEM, and any points without error bars indicate instances in which the SEM is encompassed by the data point. *Abscissae:* Dose of drug expressed as mg/kg and plotted on a logarithmic scale. The points at SAL and TD represent saline and MDMA training sessions, respectively. Filled symbols represent data from S(+)-MDMA trained mice, while open symbols represent data from R(-)-MDMA trained mice. *Ordinates:* Percent MDMA-appropriate responding (A) or response rate (B). A numeral adjacent to a symbol indicates the number of animals completing the test, if less than 6. A # indicates a significant difference from saline in the S(+)-MDMA trained group whereas an * indicates the same relationship in the R(-)-MDMA trained groups. Significant differences were assessed by one-way repeated measures analysis of variance with post-hoc analysis carried out using Dunnett's method.

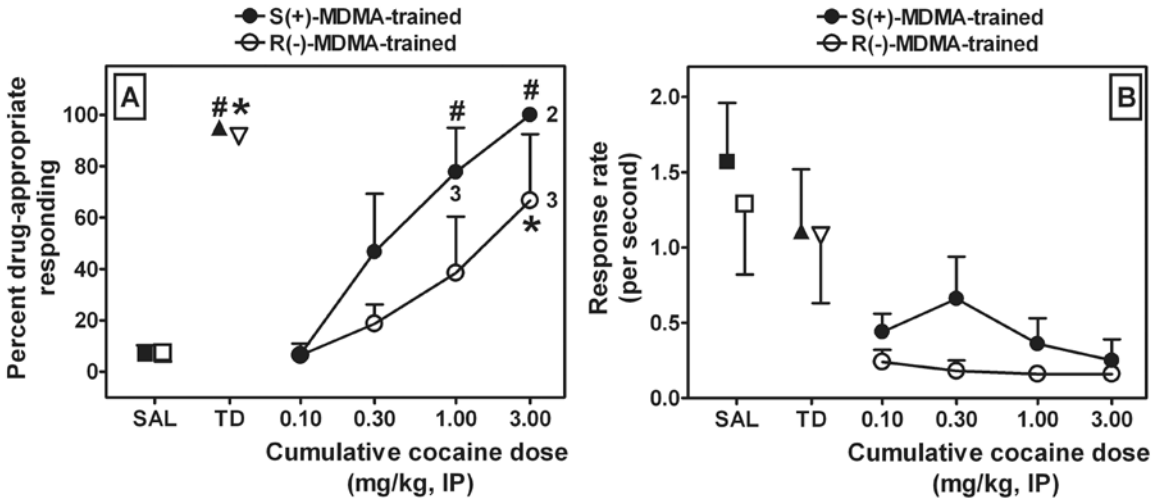


**Figure 2-3.** Effects of cocaine in mice trained with 1.5 mg/kg S(+)-MDMA (closed circles) or 1.5 mg/kg R(-)-MDMA (open circles) as a interoceptive (N=6 per group). *Abscissae and Ordinates:* as described in Figure 2-2.

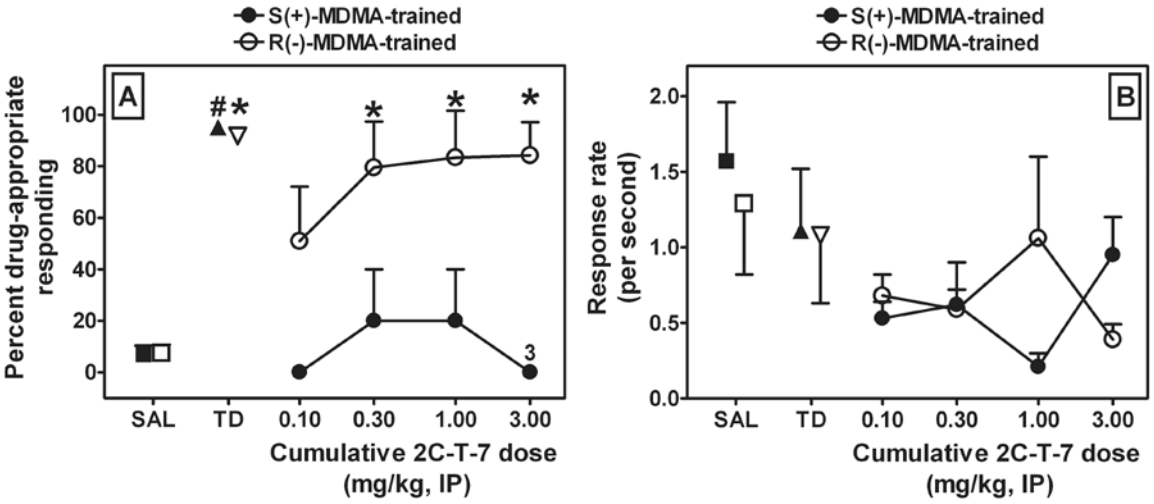


**Figure 2-4.** Effects of 2C-T-7 in mice trained with 1.5 mg/kg S(+)-MDMA (closed circles) or 1.5 mg/kg R(-)-MDMA (open circles) as a interoceptive (N=6 per group). *Abscissae and Ordinates:* as described in Figure 2-2.

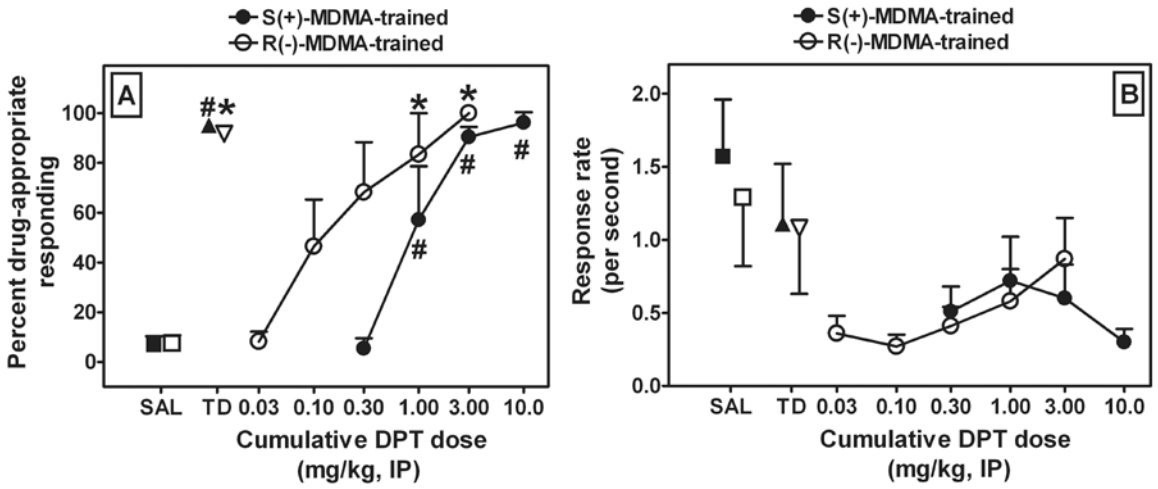


**Figure 2-5.** Effects of DPT in mice trained with 1.5 mg/kg S(+)-MDMA (closed circles) or 1.5 mg/kg R(-)-MDMA (open circles) as a interoceptive (N=6 per group). *Abscissae and Ordinates:* as described in Figure 2-2.

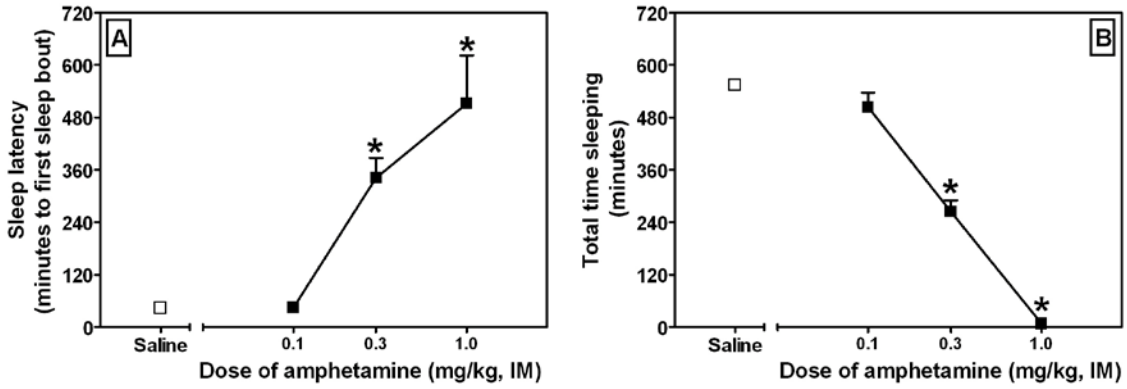


**Figure 2-6.** Effects of amphetamine on sleep in rhesus monkeys. All points represent the mean ± SEM, and any points without error bars indicate instances in which the SEM is encompassed by the data point. *Abscissae:* Dose of drug expressed as mg/kg and plotted on a logarithmic scale. The points at saline represent administration of the saline vehicle of amphetamine. *Ordinates:* Latency to the first sleep bout from the commencement of the epoch of darkness (A) or total sleep duration over the entire epoch of darkness (B). A * indicates a significant difference from saline treatment as assessed by Dunnett's method.

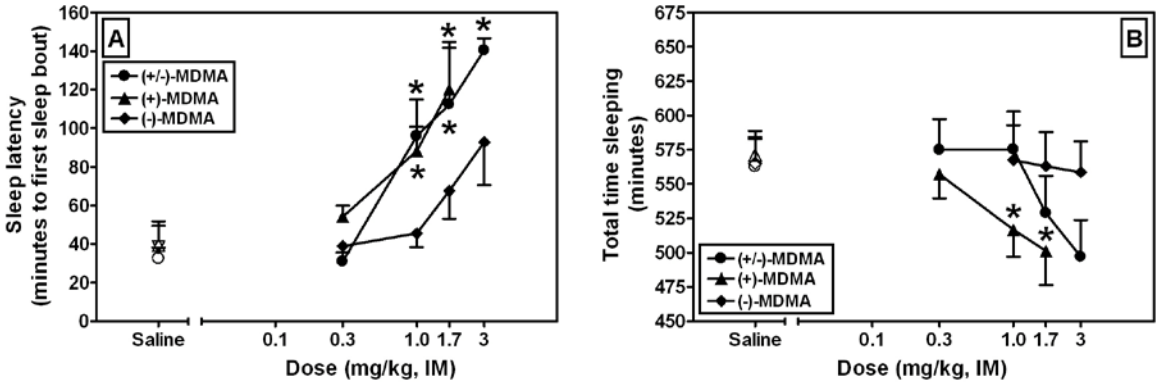


**Figure 2-7.** Effects of each form of MDMA on sleep in rhesus monkeys. *Abscissae and Ordinates:* as described in Figure 2-6.

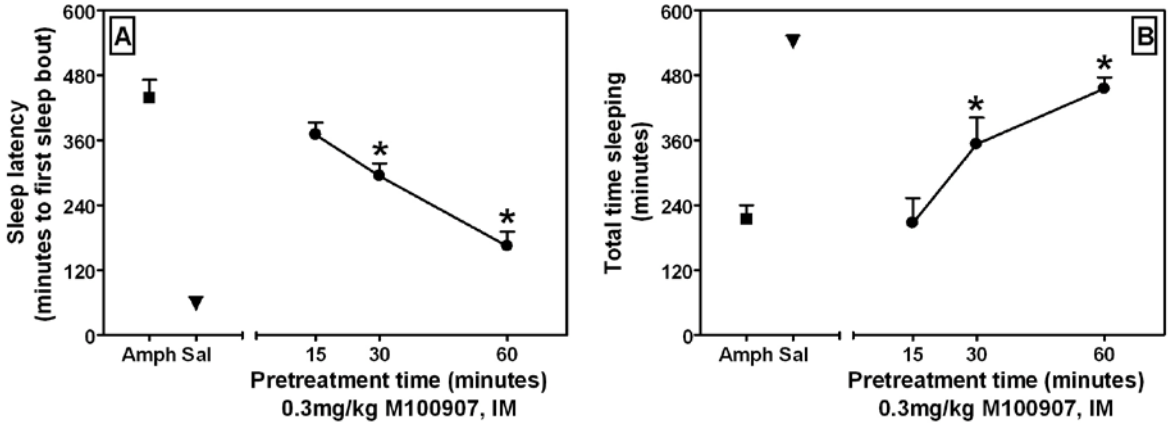


**Figure 2-8.** Influence of pretreatment time on the effects of 5-$HT_{2A}$ receptor antagonism by M100907 (0.3 mg/kg, IM) on sleep disruption by amphetamine. *Abscissae:* Dose of drug expressed as mg/kg and plotted on a logarithmic scale. The points at Amph and Sal represent administration of amphetamine or saline pretreated with the vehicle of M100907, respectively. *Ordinates:* Latency to the first sleep bout from the commencement of the epoch of darkness (A) or total sleep duration over the entire epoch of darkness (B). A * indicates a significant difference from pretreatment with vehicle as assessed by Dunnett's method.

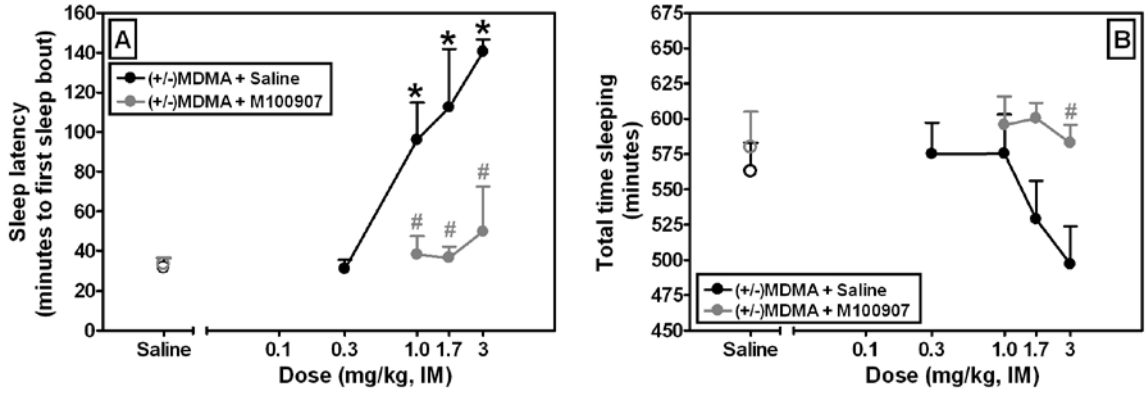


**Figure 2-9.** Effects of selective antagonism of the 5-$HT_{2A}$ receptor by M100907 (0.3 mg/kg) on the sleep-disrupting effects of S,R(+/-)-MDMA. *Abscissae and Ordinates:* as described in Figure 2-6. A * indicates a significant difference from saline treatment as assessed by Dunnett's method. A # indicates a significant difference from the same dose of MDMA following pretreatment with saline as assessed by a paired t-test.

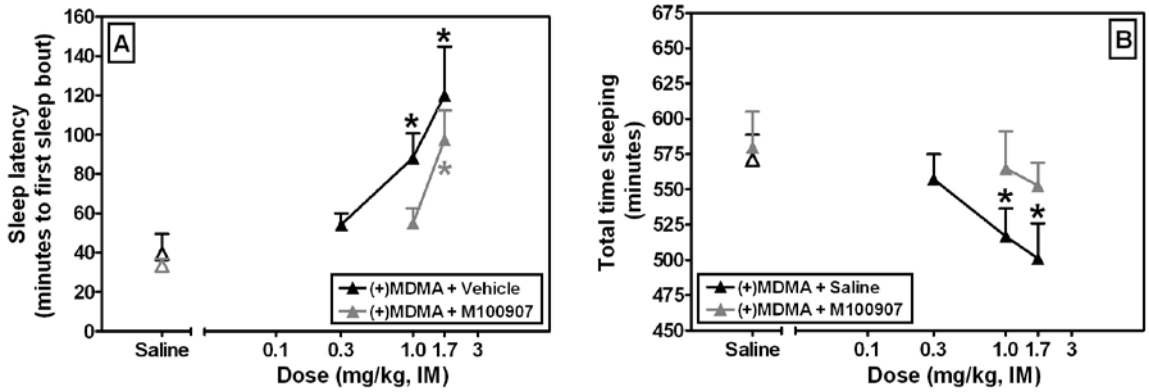


**Figure 2-10.** Effects of selective antagonism of the 5-$HT_{2A}$ receptor by M100907 (0.3 mg/kg, IM) on the sleep-disrupting effects of S(+)-MDMA. *Abscissae and Ordinates:* as described in Figure 2-6.

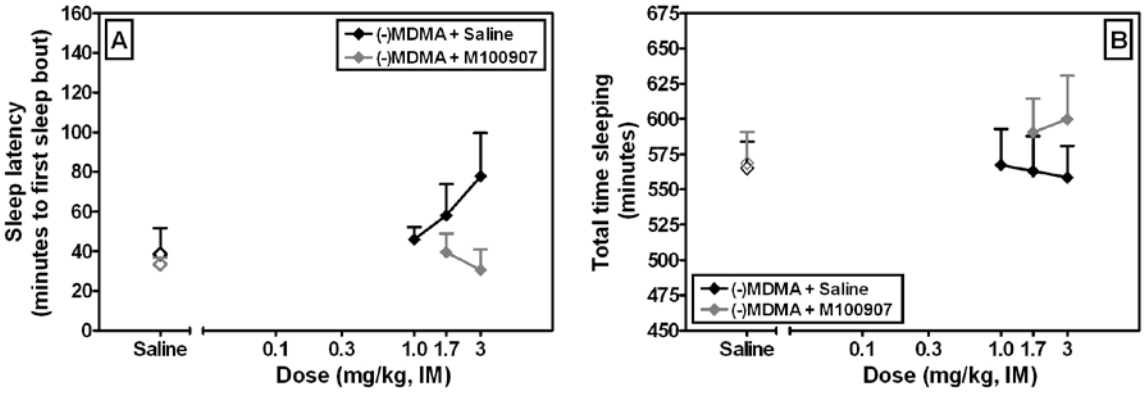


**Figure 2-11.** Effects of selective antagonism of the 5-$HT_{2A}$ receptor by M100907 (0.3 mg/kg) on the sleep-disrupting effects of R(-)-MDMA. *Abscissae and Ordinates:* as described in Figure 2-6.

# Chapter 3

## Endocrine and neurochemical effects of MDMA

Partially adapted from Murnane KS, Fantegrossi WE, Godfrey JR, Banks ML, and Howell LL “Endocrine and neurochemical effects of MDMA and its stereoisomers in rhesus monkeys” Journal of Pharmacology and Experimental Therapeutics, In press

## Introduction

Racemic 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) is a substituted phenethylamine with significant abuse liability. Although MDMA was placed into Schedule 1 of the Controled Substances Act, its behavioral effects do not clearly fit into traditional delineations of drugs of abuse. Specifically, the racemic mixture of MDMA has both stimulant and hallucinogen-like effects (Shulgin, 1986; Harris et al., 2002). Moreover, Nichols (1986) postulated that MDMA represented a new class of compounds categorized as “entactogens”. In support for this new categorization, Johanson and colleagues (2006) reported that in a three-choice discrimination procedure approximately half of the human subjects reported that MDMA was similar to the substrate-based dopamine releaser S(+)-amphetamine while the other half reported that it was similar to the serotonin releaser meta-chlorophenylpiperazine (mCPP). A thorough mechanistic understanding of the complex effects of MDMA will likely support the development of treatment strategies for reducing its abuse.

Previous studies have suggested that these complex effects are mediated by qualitative differences (i.e. apparent efficacy differences) between MDMA’s stereoisomers. For example, one of the earliest studies contrasted the subjective effects of these stereoisomers in humans (Anderson et al., 1978). This work has been supported by a series of studies utilizing drug-discrimination – the pre-clinical analogue of subjective effects in humans (Schuster and Johanson, 1988; Brauer et al., 1997) – showing marked differences in the interoceptive effects of each stereoisomer. Specifically, these studies support the notion that S(+)-

MDMA more readily functions as a psychomotor-stimulant whereas R(-)-MDMA more readily functions as a hallucinogen (Glennon et al., 1988; Baker et al., 1995; Murnane et al., 2009). This work has been further supported by studies showing that S(+)-MDMA and S,R(+/-)-MDMA, but not R(-)-MDMA, functioned as locomotor-stimulants (Fantegrossi et al., 2003) or positive reinforcers, under a progressive ratio schedule (Wang and Woolverton, 2007). Taken together, this literature suggests that the stereoisomers of MDMA have distinct behavioral and interoceptive effects. Despite these findings, other results call this hypothesis into question. For example, Taffe and colleagues (2006) found that all three forms of MDMA-elicited hyperthermia and did not function as locomotor-stimulants. Furthermore, Fantegrossi and colleagues (Fantegrossi et al., 2004b) found that all three forms of MDMA functioned as positive reinforcers, under a fixed-ratio schedule. Therefore, further study of the *in vivo* effects of these stereoisomers is warranted.

The complex biological effects of MDMA may be mediated by a number of endocrine and neurochemical effects elicited by this compound. Some of the most well established endocrine and neurochemical effects elicited by MDMA include secretion of prolactin and release of dopamine and serotonin in the central nervous system. For example, when administered 1.5 mg/kg of the racemate, human MDMA abusers not only reported complex subjective effects but also exhibited an increased level of circulating prolactin (Harris et al., 2002). As such, if the stereoisomers of MDMA have qualitatively different behavioral or interoceptive effects they should concomitantly exhibit qualitatively different

endocrine and neurochemical effects. In support of this contention, it has been shown that S(+)-MDMA increases extracellular dopamine turnover in the striatum (Hatzidimitriou et al., 2002; Acquas et al., 2007) and significantly occupies the dopamine transporter (Fantegrossi, 2008), whereas R(-)-MDMA does not. However, to date, the effects of the stereoisomers of MDMA on release of dopamine and serotonin and secretion of prolactin have not been comprehensively studied.

In addition to characterizing its underlying stereochemical basis, preclinical study of the neuropharmacology of MDMA may provide novel targets for attenuating its neurochemical effects. Since these neurochemical effects may mediate many of the behavioral and interoeceptive effects of MDMA thought to be key components of MDMA abuse, this would provide substantive support for the continued development of novel pharmacotherapeutics. Previous work suggests that the 5-$HT_{2A}$ receptor may be a viable target for attenuating MDMA abuse. Considerable evidence suggests that direct or indirect agonism of this receptor elicits hallucinogenic effects (Nichols et al., 2004). As such, antagonism of this receptor may attenuate the hallucinogenic effects of MDMA. Furthermore, this receptor may also play a critical role in the psychomotor-stimulant-like effects of MDMA as antagonism of this receptor attenuates the behavioral effects of other stimulants such as cocaine (McMahon and Cunningham, 2001) and amphetamine (Chapter 2). In addition, behavioral effects of MDMA consistent with psychomotor-stimulant-like effects such as locomotor stimulation and hyperthermia are also attenuated by antagonism of this receptor (Kehne et al.,

1996; Fantegrossi et al., 2003; Herin et al., 2005). Perhaps most promisingly, antagonism of this receptor has been shown to attenuate the sleep-disrupting effects of MDMA (Chapter 2) and MDMA self-administration (Fantegrossi et al., 2002) in rhesus monkeys. Therefore, further study of the role of this receptor in the neuropharmacology of MDMA is warranted.

Other work has implicated the SERT in many of the interoceptive and neuropharmacological effects of MDMA. For example, the subjective effects of MDMA can be attenuated by acute (Liechti et al., 2000; Liechti et al., 2001) or chronic (Stein and Rink, 1999) treatment with SSRIs. It is perhaps surprising that a drug that elevates serotonin levels, such as an SSRI, may attenuate the effects of another drug that elevates serotonin levels, such as MDMA. However, further research has suggested that this may, in fact, be mediated by an attenuation of MDMA's effects on serotonin neurotransmission. That MDMA induces a rapid and robust *in vivo* increase in extracellular serotonin levels is well established (Gough et al., 1991; Yamamoto et al., 1995; Gudelsky and Nash, 1996; Sabol and Seiden, 1998; Mechan et al., 2002; Green et al., 2003; Baumann et al., 2007). For example, one early study showed that MDMA dose-dependently increased 5-HT release in both the striatum and the pre-frontal cortex (Gudelsky and Nash 1996). This effect of MDMA on serotonin release has been shown to be diminished by acute SSRI pretreatment in either the striatum (Gudelsky and Nash 1996) or the hippocampus (Mechan et al., 2002), suggesting a parsimonious explanation for SSRI attenuation of MDMA-elicited subjective effects. However, MDMA-elicited prolactin secretion could provide an alternative

explanation as it may mediate some of the subjective effects of MDMA and may also be sensitive to SSRI pretreatment. To date, the effects of SSRI pretreatment of MDMA-elicited prolactin secretion have not been tested. Such a study would provide important new insight into the neuropharmacology of MDMA. Furthermore, it would support the development of SSRIs as potential pharmacotherapeutics for MDMA abuse as it would demonstrate which neuropharmacological effects of MDMA are likely to be attenuated by SSRIs in humans.

In summary, MDMA produces complex behavioral and interoceptive effects which may be mediated via qualitative endocrine and neurochemical differences in its stereoisomers. However, relatively little data regarding the endocrine and neurochemical effects of these stereoisomers have been published. In addition, previous work suggests that the 5-$HT_{2A}$ receptor and the SERT may be viable targets for attenuating some of these neuropharmacological effects of MDMA. Therefore, in the present study, the effects of each stereoisomer and the racemic mixture on secretion of prolactin and release of dopamine and serotonin were studied in rhesus monkeys. Circulating prolactin levels were assessed via enzyme linked immunosorbent assay. Given the novelty of these prolactin procedures in rhesus monkeys, initial experiments used amphetamine and mCPP as positive controls due to the selectivity of their monoamine releasing effects (Owens et al., 1997; Davids et al., 2002), their established effects on prolactin secretion (Muller et al., 1983; Aloi et al., 1984; Baumann et al., 2008), and the similarity of their subjective effects to MDMA

(Tancer and Johanson, 2003; Johanson et al., 2006). Furthermore, the relationship between the timecourses of MDMA-elicited serotonin release and prolactin secretion was established via concurrent determinations of plasma prolactin and striatal extracellular serotonin concentrations. In addition, the effects of each form of MDMA on striatal extracellular dopamine and serotonin levels were assessed via *in vivo* microdialysis and high pressure liquid chromatography (HPLC). Finally, experiments were carried out to establish the role of the 5-$HT_{2A}$ receptor and the SERT in the neuropharmacology of MDMA. The specific hypothesis tested was that S(+)-MDMA would function as a mixed dopamine / serotonin releaser whereas R(-)-MDMA would selectively release serotonin. As an extension of this hypothesis, we predicted that these neurochemical effects would result in R(-)-MDMA engendering more pronounced effects on prolactin secretion than S(+)-MDMA. Finally, we predicted that antagonism of the 5-$HT_{2A}$ receptor would attenuate dopamine release by MDMA and blockade of the SERT would attenuate MDMA-elicited prolactin secretion.

## Materials and methods

### *Subjects*

Four female rhesus monkeys (*Macaca mulatta*) weighing between 6.5 and 8.0 kgs served as subjects for these experiments. Subjects were housed individually within a primate colony with continuous access to water and were fed daily in the early evening after their experiments had been completed. Their diet

consisted of Purina monkey chow (Ralston Purina, St. Louis, MO) supplemented with fresh fruit and vegetables and food restriction protocols were not utilized. Ambient conditions within the colony were maintained at a temperature of 22±2°C and at 45-50% humidity; room lighting was set to a 12-h light/dark cycle. Environmental enrichment was provided on a regular basis. All procedures and studies strictly followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee of Emory University.

*Surgery*

Prior to initiation of this study, subjects were implanted with chronic indwelling venous catheters using aseptic surgical techniques as previously described (Wilcox et al., 2002). Subjects were also implanted with bilateral CMA/11 guide cannulae (CMA, North Chelmsford, MA, USA) that were stereotaxically targeted for the head of the caudate nucleus as previously described (Czoty et al., 2000; Wilcox et al., 2005). During each surgery, subjects were prophylactically administered an antibiotic (Rocephin), an analgesic (Buprenorphine), and a nonsteroidal anti-inflammatory agent (Banamine) to minimize any pain or discomfort that may result from the surgery. Catheters were regularly flushed with heparinized (100 U/ml) saline to maintain patency.

*Drugs*

S(+), R(-), and S,R(+/-)-methylenedioxymethamphetamine (Figure 1) were supplied by the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC). S(+)-amphetamine, *Meta*-chlorophenylpiperazine (mCPP), and fluoxetine were commercially purchased from Sigma-Aldrich (St. Louis, MO). M100907 was synthesized at the Laboratory of Medicinal Chemistry at the National Institutes of Diabetes, Digestive and Kidney Disorders at the National Institutes of Health (Bethesda, MD) and was a generous gift from Dr. Kenner C. Rice. Doses were calculated and are expressed as salts. All drugs were dissolved in 0.9% sterile saline and administered intraveneously. Where possible, the doses of each compound were chosen on the basis of positive results, under similar conditions, from previous internal studies or the peer-reviewed literature.

*Procedure*

Dosing schedule and drug history

All procedures were carried out in fully conscious subjects while they sat in commercially available primate chairs (Primate Products, Woodside, CA). All subjects had a history of contingent and noncontingent administration of both cocaine and MDMA prior to the initiation of this study. The order of drug administration was randomized without regard to which assay was carried out on a particular day and there was a minimum of five days between sessions. The doses studied were chosen on the basis that they are equivalent, on a mg/kg

basis, to the doses of MDMA typically abused by humans (Cole et al., 2002; Harris et al., 2002; Green et al., 2003) and are within the range that rhesus monkeys will voluntarily self-administer (Fantegrossi et al., 2002; Banks et al., 2008). However, the first time 3.0 mg/kg of S(+)-MDMA was administered, the subject presented acute symptoms of untoward effects and it was decided that this dose of S(+)-MDMA would not be repeated due to ethical and safety concerns. The randomized dose order was thus continued while omitting any administrations of this dose of S(+)-MDMA. Plasma prolactin collection and microdialysis procedures were alternated such that a given assay was carried out every other week. Due to the need to develop new HPLC procedures (see HPLC subsection), the effects of each form of MDMA on serotonin release were determined subsequent to the experiments determining the effects of each form of MDMA on dopamine release. Likewise, determinations of the role of the 5-$HT_{2A}$ receptor and the SERT in the neuropharmacology of MDMA were carried out subsequent to all other experiments and as such were not a part of the randomized dosing order.

Plasma prolactin collection

Subjects were seated in restraint chairs and acclimated to having an acute catheter (BD Saf-T-Intima Closed Catheter System, Franklin Lakes, NJ) unilaterally inserted into a saphenous vein prior to experimental data collection. For this assay, drug administration and plasma collection were carried out using an acute catheter, rather than the chronic indwelling catheter, because a sufficient number of blood samples could not be reliably withdrawn from the

chronic catheter in all subjects. During experiments, 2.0 ml of blood were collected 15 min prior to an IV drug injection and 15, 30, 60, and 120 min after the injection. Samples were refrigerated in 3.5 ml serum separating vacutainers (BD, Franklin Lakes, NJ), centrifuged (at 3000 rpm for 15 min) to isolate the plasma, and frozen in a cryogenic freezer at -20° C (range -15° to -25° C) until they were assayed. Samples were assayed by the Yerkes National Primate Research Center's Biomarkers Core Laboratory using a fluorescence based enzyme linked immunosorbent assay (UV-ELISA) as previously described (Mook et al., 2005).

*In vivo* microdialysis

Microdialysis measurements were collected and samples analyzed in a similar fashion to procedures that have been previously described (Kimmel et al., 2007; Banks et al., 2009). Briefly, subjects were placed in sound attenuated testing chambers after 24 mm stainless steel microdialysis probes with a 4 mm membrane (CMA/Microdialysis) were inserted into the subject's surgically implanted guide cannulae. Drugs were administered through the previously implanted indwelling venous catheter via the subcutaneous vascular access port. Experiments consisted of a 1 h equilibrium period after which samples were collected every 10 min for 3 h. Drugs were administered 1 h after the sampling phase initiated in order to provide a pre- and post-drug sampling period. Probe function was verified for each experimental session, both pre- and post-session, via determination of the change in neurotransmitter concentration as a function of traversing the probe with a known concentration of dopamine (i.e. probe

recovery). Furthermore, the viability of the sampling site was verified through retrodialysis of a potassium-enriched (100 mM) solution ionically matched to cerebrospinal fluid (aCSF). Neurochemical concentrations within the dialysate were quantified via electrochemical detection utilizing high pressure liquid chromatography (HPLC) and analyzed in comparison to known concentration curves with EZChrom Elite software (version 3.1, Scientific Software, Pleasanton, CA).

*HPLC*

High pressure liquid chromatography and electrochemical detection were used to quantify dopamine levels as previously described (Kimmel et al., 2007; Banks et al., 2009). Briefly, the HPLC system was composed of a small bore column (3.2 mm X 150 mm X 3 µm), an ESA 582 model solvent delivery pump set to a flow rate of 0.6 ml/min, and a ESA model 542 autosampler (ESA, Inc., Chelmsford, MA). Electrochemical detection was carried out via a guard cell (ESA model 5020, potential = 350 mV), a dual channel analytical cell (ESA model 5040), and an ESA model Coulochem II detector. The analytical cell's oxidative channel was set to a voltage of –150 mV and its reductive channel was set to 275 mV. Different commercially available mobile phases were used for dopamine (MD-TM, ESA, Inc.) or serotonin (MD-TM2, ESA, Inc.) quantification. MD-TM is composed of sodium dihydrogen phosphate (75 mM), octanesulfonic acid (1.7 mM), triethylamine (TEA, 100 µL / L), EDTA (25 µM), and acetonitrile (10%); upon mixing, it is brought to a final pH of 3 via addition of phosphoric acid. MD-TM2 is composed of sodium dihydrogen phosphate (90 mM), octanesulfonic acid

(1.7 mM), citric acid (50 mM), EDTA (50 μM), and acetonitrile (10%); upon mixing, it is brought to a final pH of 3 via addition of phosphoric acid. All other microdialysis and HPLC procedures were identical to those used for dopamine quantification.

*Data Analysis*

Graphical presentation of all data depicts mean ± SEM, and any points without error bars indicate instances in which the SEM is encompassed by the data point or data derived from a single subject. All graphical data presentations were created using GraphPad Prism 4 (La Jolla, CA), all statistical tests were performed using SigmaStat 3 (San Jose, CA), and significance was arbitrated at a $p<0.05$.

Plasma prolactin analysis

The primary dependent variable tested in this experiment was plasma concentration of prolactin. Differences in basal levels across days were assessed via a one-way repeated measures (RM) analysis of variance (ANOVA). Data were then normalized to the measured baseline levels in a given experimental session. Main effects of condition and time were analyzed via a two-way RM ANOVA. Individual comparisons were then drawn at each time point with correction for multiple comparisons utilizing Dunnett's method versus baseline levels. Assessment of the effects of MDMA administration on basal prolactin levels (basal levels during the first or final prolactin determination) were analyzed

using a paired t-test. Pearson correlation analysis was used to determine the relationship between MDMA-elicited serotonin release and prolactin secretion.

Neurochemical analysis

The primary dependent variable tested in this experiment was striatal extracellular concentration of the neurotransmitters serotonin or dopamine. For dopamine, differences in basal levels across days were assessed via a one-way RM ANOVA. Assessment of the effects of MDMA administration on basal levels without correction for probe recovery of dopamine and its major metabolites DOPAC and HVA (basal levels during the first or final microdialysis determination; Table 1) were analyzed using a paired t-test. Basal levels of extracellular serotonin in the three experimental sessions carried out to determine the effects of each form of MDMA on this neurochemical were analyzed via a one-way RM ANOVA. Dopamine and serotonin data were then normalized to the measured baseline levels in a given experimental session. The main effect of treatment was analyzed via a one-way RM ANOVA. Individual comparisons were then drawn at each time point with correction for multiple comparisons utilizing Tukey's test versus baseline levels. Pretreatment effects of the selective 5-$HT_{2A}$ receptor antagonist M100907 or the SSRI fluoxetine were analyzed via a two-way RM ANOVA with the main factors of pretreatment and time were analyzed. Across time, individual comparisons were then drawn at each time point with correction for multiple comparisons utilizing Dunnett's method versus baseline levels. Across pretreatment, individual comparisons were carried out via a paired t-test.

## Results

### Basal endocrine and neurochemical levels

*Basal levels of prolactin, dopamine, DOPAC, and HVA (Table 3-1)*

Table 1 shows the basal hormone and neurochemical levels determined during the first or last endocrine (prolactin) or dopamine microdialysis (dopamine, DOPAC, and HVA) experiment in each subject. Analysis by paired t-test revealed that there were no significant differences in basal prolactin ($t_3$ = -2.21; p = 0.114), dopamine ($t_3$ = 0.722; p = 0.114), DOPAC ($t_3$ = 1.487; p = 0.234), or HVA ($t_3$ = -0.385; p = 0.726) levels across these time points. However, the power of each test was only 0.28, 0.52, 0.12, or 0.52, respectively.

### Effects on circulating prolactin levels

*Amphetamine vs mCPP (Figure 3-2)*

Under the procedures employed, circulating levels of plasma prolactin could be reliably obtained from rhesus monkeys. A two-way RM ANOVA revealed a significant main effect of both drug ($F_{3,2}$ = 16.656; p = 0.004) and time ($F_{3,4}$ = 12.614; p <0.001) and a significant interaction ($F_8$ = 12.988; p <0.001). The power of this test for the main effect of drug was 0.974, of time was 0.998, and of the interaction was 1. Basal levels of prolactin did not differ depending on the day of the treatment as determined via a one-way repeated measures analysis of variance ($F_{3,2}$ = 1.457; p = 0.305). However, the power of this test was only 0.095. Post-hoc analysis by means of Dunnett's test showed that saline

administration did not significantly alter circulating prolactin compared to baseline at any time point ($F_{3,4}$ = 0.720; p = 0.595). However, intravenous administration of 2.5 mg/kg of mCPP, used as a positive control for the effects of a serotonin releaser, significantly elevated prolactin ($F_{3,4}$ = 12.934; p < 0.001) at 15, 30, and 60 min (p < 0.05) but not at 120 min. In contrast, intravenous administration of 1.0 mg/kg of (+)-amphetamine, used as a positive control for the effects of a dopamine releaser, significantly decreased prolactin levels ($F_{3,4}$ = 9.150; p = 0.001) at 30, 60, and 120 min (p < 0.05) but not at 15 min.

*Correlation between MDMA-elicited serotonin release and prolactin secretion (Figure 3-3)*

Concurrent microdialysis and blood collection was carried out to determine the relationship between MDMA-elicited serotonin release and prolactin secretion. Pearson correlation analysis revealed that there was a significant relationship between striatal extracellular serotonin levels and plasma prolactin concentrations ($r^2$ = 0.850; p < 0.0001) following administration of S,R(+/-)-MDMA at 1.7 mg/kg. Furthermore, there was also a significant relationship between striatal extracellular levels of the major serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) and plasma prolactin concentrations ($r^2$ = 0.485; p = 0.01; data not shown).

*MDMA stereoisomers (Figure 3-4)*

One-way RM ANOVA revealed that basal levels of prolactin did not differ across day ($F_{3,9}$ = 1.771; p = 0.121), however the power of this test was only

0.292. Two-way RM ANOVA showed a significant main effect of treatment (drug and dose; $F_{3,7} = 5.585$; $p < 0.001$), time ($F_{3,4} = 11.432$; $p < 0.001$), and a significant interaction ($F_{28} = 5.315$; $p < 0.001$). The power of this test for the main effect of treatment was 0.966, of time was 0.996, and of the interaction was 1. The 3.0 mg/kg S(+)-MDMA data were not included in this analysis because they were derived from a single subject (see Methods). Post-hoc analysis by means of Dunnett's test showed that, at 15 min, there was a significant effect of treatment ($F_{3,8} = 6.641$; $p < 0.001$) that was exclusively due to the effects of 1.7 mg/kg R(-)-MDMA ($p < 0.05$). At 30 min a significant main effect remained ($F_{3,8} = 6.289$; $p < 0.001$) but treatment isolation via Dunnett's method showed that this was due to both 1.7 mg/kg R(-)-MDMA and 1.7 mg/kg S,R(+/-)-MDMA ($p < 0.05$). A significant main effect was also found at 60 min ($F_{3,8} = 2.693$; $p < 0.031$) due to the effects of 1.7 mg/kg R(-)-MDMA ($p < 0.05$). No significant main effect of treatment was found at 120 min ($F_{3,8} = 1.122$; $p = 0.387$). In contrast to these effects of R(-)-MDMA, S(+)-MDMA had no significant effect compared to baseline on prolactin levels at any of the measured timepoints.

Effects on neurochemical levels

*Serotonergic effects at 1.7 mg/kg (Figure 3-5)*

Under the procedures employed, measurements of extracellular levels of serotonin in the caudate could be reliably obtained from rhesus monkeys. Basal extracellular serotonin levels uncorrected for probe recovery were 0.47 ± 0.27, 0.15 ± 0.02, and 0.29 ± 0.14 during the sessions carried out to determine the

effects of S(+)-MDMA, R(-)-MDMA, and S,R(+/-)-MDMA, respectively. One-way RM ANOVA revealed that basal extracellular serotonin levels did not differ by session ($F_{3,2}$ = 0.741; p = 0.516), however the power of this test was only 0.052. One-way RM ANOVA also showed that S(+)-MDMA ($F_{3,8}$ = 6.438; p < 0.001), S,R(+/-)-MDMA ($F_{3,8}$ = 4.868; p < 0.001), and R(-)-MDMA ($F_{3,8}$ = 2.864; p = 0.022) significantly elevated extracellular serotonin levels. Post-hoc analysis via the Tukey's test showed that these effects were significant for all three compounds at the 20 min time point.

*Dopaminergic effects at 1.7 mg/kg (Figure 3-6)*

Under the procedures employed, measurements of extracellular levels of dopamine in the caudate could be reliably obtained from rhesus monkeys. One-way RM ANOVA showed that both S(+)-MDMA ($F_{3,8}$ = 3.232; p = 0.012) and S,R(+/-)-MDMA ($F_{3,8}$ = 5.027; p < 0.001) significantly elevated dopamine levels. Post-hoc analysis via the Tukey's test showed that these effects were significant for both compounds at the 20 min time point. In contrast, R(-)-MDMA had no significant effects compared to baseline on extracellular dopamine levels ($F_{3,8}$ = 0.884; p = 0.544) analyzed across the first 60 min after drug administration.

*Dose-effect determination of R(-)-MDMA (Figure 3-7)*

To ascertain whether the differential effects of S(+)-MDMA and R(-)-MDMA, shown in Figure 3-6, were due to a potency difference, 3 mg/kg of R(-)-MDMA was administered to determine if this stereoisomer would produce a change in extracellular dopamine levels at a higher dose. One-way repeated

measures analysis of variance revealed no significant main effect of treatment ($F_{3,8}$ = 1.963; p = 0.098) with this dose of R(-)-MDMA. Higher doses were not tested due to safety concerns regarding the effects of R(-)-MDMA on heart rate (Fantegrossi, 2008) and the presentation of acute untoward effects of S(+)-MDMA at 3 mg/kg in this study.

*In vivo interaction: 1.7 mg/kg (+/-)-MDMA vs 0.85 mg/kg (+)-MDMA (Figure 3-8)*

To determine whether co-administration of R(-)-MDMA with S(+)-MDMA, as in the racemic mixture, potentiates or diminishes the dopaminergic effects of S(+)-MDMA, the effects of 1.7 mg/kg S,R(+/-)-MDMA were compared to those of 0.85 mg/kg S(+)-MDMA. Since the racemate is composed of equal parts of the two stereoisomers, this equalized the amount of S(+)-MDMA administered. Two-way repeated measures analysis of variance revealed a significant main effect of treatment with these compounds at these doses ($F_{3,8}$ = 5.460; p < 0.001) but no significant difference between them ($F_{3,1}$ = 0.610; p = 0.492).

*Effects of 5-$HT_{2A}$ antagonism on basal levels of dopamine (Figure 3-9)*

Additional experiments were undertaken to determine the effects of antagonism of the 5-$HT_{2A}$ receptor on dopamine release. To ensure that any effects on dopamine release were not due to alterations of basal dopamine levels, the effects of 5-$HT_{2A}$ antagonism by 0.3mg/kg of M100907 on basal levels of dopamine were evaluated. A two-way RM ANOVA revealed no significant main effect of either pretreatment ($F_{3,1}$ = 2.205; p = 0.234) or time ($F_{3,4}$ = 1.147; p = 0.379) and no significant interaction ($F_8$ = 1.809; p = 0.171). The power of this

test for the main effect of pretreatment was 0.124, of time was 0.078, and of the interaction was 0.212.

*Effects of 5-$HT_{2A}$ antagonism on amphetamine elicited dopamine release (Figure 3-10)*

The effects of antagonism of the 5-$HT_{2A}$ receptor antagonism by 0.3mg/kg of M100907 on dopamine release by amphetamine (0.3 mg/kg or 1.0 mg/kg) were then evaluated. A two-way RM ANOVA revealed a significant main effect of treatment with amphetamine at 0.3 mg/kg ($F_{3,12}$ = 3.309; p = 0.003) but no main effect of pretreatment with M100907 ($F_{3,1}$ = 3.200; p = 0.172) and no significant interaction ($F_{12}$ = 1.635; p = 0.125). The powers of these tests were 0.891, 0.183, and 0.288, respectively. Post-hoc analysis revealed a significant effect of amphetamine following pretreatment with vehicle ($F_{3,12}$ = 2.501; p = 0.017) and that dopamine levels following this treatment were significantly different from baseline at 30 and 40 minutes. Following pretreatment with M100907, these was no significant main effect of treatment with amphetamine ($F_{3,12}$ = 1.620; p = 0.130). The powers of these tests were 0.678 and 0.281, respectively. Furthermore, a two-way RM ANOVA revealed a significant main effect of treatment with amphetamine at 1.0 mg/kg ($F_{3,12}$ = 6.924; p < 0.001), pretreatment with M100907 ($F_{3,1}$ = 20.013; p = 0.021), and a significant interaction ($F_{12}$ = 2.870; p = 0.007). The powers of these tests were 1.000, 0.821, and 0.793, respectively. Post-hoc analysis revealed a significant effect of amphetamine following pretreatment with vehicle ($F_{3,12}$ = 2.501; p = 0.017) and that dopamine levels following this treatment were significantly different from baseline at 30 and

40 minutes. Following pretreatment with M100907, these was no significant main effect of treatment with amphetamine ($F_{3,12}$ = 1.620; p = 0.130). The powers of these tests were 0.678 and 0.281, respectively.

*Effects of 5-$HT_{2A}$ antagonism on S(+)-MDMA-elicited dopamine release (Figure 3-11)*

To determine if the capacity of 5-$HT_{2A}$ receptor antagonism to attenuate amphetamine elicited dopamine release generalized to MDMA, dopamine release by S(+)-MDMA (1.7 mg/kg) was then evaluated following pretreatment by vehicle or M100907 (0.3 mg/kg). Initial experiments showed that M100907 pretreatment was ineffective when administered 30 minutes prior to administration of S(+)-MDMA (data not shown). Therefore, the effects of M100907 were reevaluated with a 60 minute pretreatment time as longer pretreatment times were shown to be more effective in the behavioral experiments described in chapter 2. A two-way RM ANOVA revealed a significant main effect of treatment with S(+)-MDMA ($F_{3,6}$ = 9.874; p < 0.001), no main effect of pretreatment with M100907 ($F_{3,1}$ = 6.345; p = 0.086), but a significant interaction ($F_6$ = 6.136; p = 0.001). The powers of these tests were 0.999, 0.359, and 0.963, respectively. Post-hoc analysis revealed a significant effect of S(+)-MDMA following pretreatment with vehicle ($F_{3,6}$ = 8.469; p < 0.001) and that dopamine levels following this treatment were significantly different from baseline at 10, 20, and 30 minutes. Following pretreatment with M100907, there was no significant main effect of treatment with S(+)-MDMA ($F_{3,6}$ = 2.469; p = 0.100). The powers of these tests were 0.997 and 0.642, respectively.

*Effects of fluoxetine on S,R(+/-)-MDMA-elicited prolactin secretion (Figure 3-12)*

Next, the role of the SERT in prolactin secretion elicited by S,R(+/-)-MDMA (1.7 and 3.0 mg/kg) was evaluated by pretreatment with the SSRI fluoxetine (3.0 mg/kg). A two-way RM ANOVA revealed a significant main effect of both treatment with S,R(+/-)-MDMA ($F_{3,5}$ = 8.267; $p < 0.001$) and pretreatment with fluoxetine ($F_{3,1}$ = 12.565; $p = 0.038$) and a significant interaction ($F_5$ = 14.962; $p < 0.001$). The powers of each of these tests were 0.987, 0.630, and 1.000, respectively. Post-hoc analysis revealed a significant effect of S,R(+/-)-MDMA following pretreatment with vehicle ($F_{3,5}$ = 10.448; $p < 0.001$) and that prolactin levels following this treatment were significantly different from baseline at 30 and 60 minutes. Following pretreatment with fluoxetine, these was also a significant main effect of treatment with S,R(+/-)-MDMA ($F_{3,5}$ = 6.404; $p = 0.002$); however, no time point was significantly different from baseline. The powers of these tests were 0.998 and 0.942, respectively. Post-hoc analysis via a paired t-test revealed that following pretreatment with either fluoxetine or its vehicle the effects of S,R(+/-)-MDMA on plasma prolactin levels were significantly different at 30 ($t_4$ = 4.785; $p = 0.017$) and 60 minutes ($t_4$ = 4.401; $p = 0.022$). Furthermore, fluoxetine had no significant effect on prolactin levels in its own right as prolaction levels were not significantly different, following administration of fluoxetine but prior to administration of MDMA, at time point 0 ($t_4$ = -2.317; $p = 0.103$). The powers of these tests were 0.866, 0.809, and 0.307, respectively. The effects of S,R(+/-)-MDMA following pretreatment with fluoxetine were then evaluated at 3.0 mg/kg of S,R(+/-)-MDMA in order to determine if at a higher dose MDMA would

surmount the effects of fluoxetine. One-way RM ANOVA revealed a significant main effect of S,R(+/-)-MDMA ($F_{3,5}$ = 7.767; p < 0.001) despite pretreatment with fluoxetine. The power of this test was 0.980. Post-hoc comparisons showed that prolactin levels were significantly different from baseline at 15 and 30 minutes (p < 0.050) following this treatment.

*Effects of fluoxetine on R(-)-MDMA-elicited prolactin secretion (Figure 3-13)*

The role of the SERT in prolactin secretion elicited by R(-)-MDMA (1.0 and 1.7 mg/kg) was then evaluated via the same pretreatment regimen used to determine its role in S,R(+/-)-MDMA-elicited prolactin secretion. At 1.0 mg/kg of R(-)-MDMA, a two-way RM ANOVA revealed a significant main effect of both treatment with R(-)-MDMA ($F_{3,5}$ = 6.745; p = 0.349) but not pretreatment with fluoxetine ($F_{3,1}$ = 0.001; p = 0.969) and no significant interaction ($F_5$ = 1.215; p = 0.349). The powers of these tests were 0.955, 0.052, and 0.086, respectively. Post-hoc analysis by means of Dunnett's test showed that there was a significant effect of R(-)-MDMA following pretreatment with vehicle ($F_{3,5}$ = 8.665; p < 0.001) and that prolactin levels were significantly different from baseline at 15 and 30 minutes (p < 0.05) following this treatment. The same analysis procedure revealed no significant main effect of R(-)-MDMA following pretreatment with fluoxetine ($F_{3,5}$ = 2.905; p = 0.050). The powers of these tests were 0.991 and 0.480, respectively. At 1.7 mg/kg of R(-)-MDMA, a two-way RM ANOVA revealed a significant main effect of both treatment with R(-)-MDMA ($F_{3,5}$ = 11.204; p < 0.001) but not pretreatment with fluoxetine ($F_{3,1}$ = 0.110; p = 0.762) and no significant interaction ($F_5$ = 0.528; p = 0.752). The powers of these tests were

0.999, 0.052, and 0.050, respectively. Post-hoc analysis by means of Dunnett's test showed that there was a significant effect of R(-)-MDMA following pretreatment with vehicle ($F_{3,5}$ = 4.062; $p < 0.016$) and that prolactin levels were significantly different from baseline at 15 ($p < 0.05$) following this treatment. The same analysis procedure revealed a significant main effect of R(-)-MDMA following pretreatment with vehicle ($F_{3,5}$ = 29.680; $p < 0.001$) and that prolactin levels were significantly different from baseline at 15 and 30 minutes ($p < 0.05$) following this treatment. The powers of these tests were 0.715 and 1.000, respectively.

*Effects of fluoxetine on R(-)-MDMA-elicited serotonin release (Figure 3-14)*

Since fluoxetine pretreatment did not significantly alter prolactin secretion elicited by R(-)-MDMA (Figure 3-13), further microdialysis experiments were undertaken to determine if fluoxetine attenuated serotonin release by R(-)-MDMA. This was deemed necessary becuase previous studies have shown that fluoxetine attenuates serotonin release by S,R(+/-)-MDMA (Gudelsky and Nash 1996; Mechan et al., 2002), however, similar studies have not been undertaken with R(-)-MDMA. A two-way RM ANOVA revealed a significant main effect of treatment with R(-)-MDMA ($F_{3,6}$ = 4.292; $p = 0.007$), no significant main effect of pretreatment with fluoxetine ($F_{3,1}$ = 8.905; $p = 0.058$), but a significant interaction ($F_6$ = 4.300; $p = 0.007$). The powers of these tests were 0.820, 0.485, and 0.821, respectively. Post-hoc analysis revealed a significant effect of R(-)-MDMA following pretreatment with vehicle ($F_{3,6}$ = 4.305; $p = 0.007$) and that prolactin levels following this treatment were significantly different from baseline at 20

minutes. Following pretreatment with fluoxetine, these was no significant main effect of treatment with R(-)-MDMA ($F_{3,6}$ = 2.169; p = 0.010). The powers of these tests were 0.822 and 0.563, respectively.

*Effects of 5-$HT_{2A}$ antagonism on R(-)-MDMA-elicited prolactin secretion (Figure 3-15)*

Since fluoxetine pretreatment attenuated R(-)-MDMA-elicited serotonin release (Figure 3-14), but not prolactin secretion (Figure 3-13), this suggests R(-)-MDMA can elicit prolactin secretion through a mechanism that does not require the SERT. Previous studies have suggested that R(-)-MDMA can function as a direct agonist of the 5-$HT_{2A}$ receptor (Nash et al., 1994; Fantergrossi et al., 2005) and that administration of agonists at this receptor can elicit prolactin secretion (Aulakh et al., 1994). Therefore, the effects of 5-$HT_{2A}$ receptor antagonism by 0.3mg/kg of M100907 on R(-)-MDMA-elicited prolactin secretion were evaluated. A two-way RM ANOVA revealed a significant main effect of both treatment with R(-)-MDMA ($F_{3,5}$ = 5.660; p = 0.004), but no main effect of pretreatment with M100907 ($F_{3,1}$ = 0.010; p = 0.925) and no significant interaction ($F_5$ = 1.059; p = 0.421). The powers of these tests were 0.899, 0.052, and 0.059, respectively. Following pretreatment with M100907, post-hoc analysis revealed no significant effect of R(-)-MDMA ($F_{3,5}$ = 2.573; p = 0.071). The power of this test was 0.400.

*Effects of combined 5-$HT_{2A}$ antagonism and fluoxetine administration on R(-)-MDMA-elicited prolactin secretion (Figure 3-16)*

Finally, experiments were carried out to determine if combined pretreatment with M100907 and fluoxetine (at the same doses previously tested) would effectively antagonize R(-)-MDMA-elicited prolactin secretion. A two-way RM ANOVA revealed a significant main effect of treatment with R(-)-MDMA ($F_{3,5}$ = 9.710; $p < 0.001$), a significant main effect of combined pretreatment with M100907 and fluoxetine ($F_{3,5}$ = 3.173; p = 0.031), but no significant interaction ($F_5$ = 1.445; p = 0.265). The powers of these tests were 0.996, 0.322, and 0.131, respectively. Post-hoc analysis revealed no significant effect of treatment with R(-)-MDMA ($F_{3,5}$ = 2.594; p = 0.070) following combined pretreatment with M100907 and flouxetine. The power of this test was 0.405. Post-hoc analysis via a paired t-test revealed a significant difference due to pretreatment at 15 ($t_4$ = 4.261; p = 0.012) and 30 minutes ($t_4$ = 4.341; p = 0.031). Furthermore, this combined pretreatment had no significant effect on prolactin levels in its own right as prolaction levels were not significantly different at time point 0 ($t_4$ = -1.317; p = 0.243). The powers of these tests were 0.842, 0.871, and 0.452, respectively.

**Discussion:**

The major finding of this study is that the stereoisomers of MDMA have distinct endocrine and neurochemical effects which are consistent with S(+)-MDMA functioning as a mixed substrate-based dopamine / serotonin releaser

while R(-)-MDMA selectively releases serotonin in rhesus monkeys. In the first experiment, the serotonin releaser mCPP (Owens et al., 1997) significantly increased circulating prolactin levels whereas the dopamine releaser (+)-amphetamine (Davids et al., 2002) significantly decreased prolactin levels. This effect is consistent with previous reports that serotonin releasers (Aloi et al., 1984; Baumann et al., 2008) and direct serotonin receptor agonists (Aulakh et al., 1994) elicit an increase in circulating prolactin and that this prolactin increase can be blocked by multiple serotonin receptor antagonists (Aulakh et al., 1994; Meltzer and Maes, 1995). Furthermore, dopamine releasers and direct dopamine receptor agonists have been shown to lower circulating prolactin levels and antagonists of dopamine receptors increase prolactin levels (Muller et al., 1983). This initial experiment was corroborated by determination of the relationship between MDMA-elicited serotonin release and prolactin secretion. These positive control experiments extend previous findings by showing prolactin secretion in rhesus monkeys is also controlled by relative dopaminergic versus serotonergic tone.

At the doses tested, administration of the stereoisomers of MDMA-elicited differential effects on prolactin levels. Previous studies have used variants of allometric scaling to equilibrate the doses of MDMA administered to experimental animals to human consumption (Mueller et al., 2008). However, these allometric models are sensitive to the pharmacokinetic and corrective factors employed and can thus yield highly variable estimates (Yates and Kugler, 1986; Fantegrossi, 2007). Furthermore, previous studies have shown that the doses of MDMA

voluntarily self-administered by rhesus monkeys (Fantegrossi et al., 2002; Banks et al., 2008) are within the range, on a mg/kg basis, that humans abuse (1 – 2 mg/kg) (Cole et al., 2002; Harris et al., 2002; Green et al., 2003). Therefore, we chose to forgo potentially problematic allometric scaling procedures in favor of simply administering doses voluntarily self-administered by human and nonhuman primates. However, it may be important to note that human MDMA abusers typically consume MDMA orally which likely yields different pharmacokinetics than the intravenous route used in this study. Nevertheless, unlike R(-)-MDMA and S,R(+/-)-MDMA, S(+)-MDMA did not significantly alter circulating prolactin levels up to a dose of 3.0 mg/kg. Importantly, the effects of S,R(+/-)-MDMA were intermediate to the effects of its two component stereoisomers and the effects of R(-)-MDMA and S,R(+/-)-MDMA on circulating levels of prolactin are consistent with those of other serotonin releasing drugs.

The results of the microdialysis experiments complement those obtained in the prolactin experiments and further support the hypothesis that S(+)-MDMA functions as a mixed substrate-based dopamine / serotonin releaser while R(-)-MDMA selectively releases serotonin. The failure of S(+)-MDMA to alter plasma prolactin could be due to two possibilities. First, S(+)-MDMA may simply not function as a serotonin releaser in rhesus monkeys and therefore would not be expected to elicit a prolactin response or alternatively, S(+)-MDMA may function as a serotonin releaser but have a more complex pharmacology than a drug that selectively releases serotonin, such as mCPP. In this regard, a complex mechanism involving released dopamine might be predicted to functionally

antagonize the effects of released serotonin on prolactin secretion. To determine which alternative best fits the data, HPLC and microdialysis procedures were used to determine the effects of S(+)-MDMA on extracellular serotonin and dopamine levels. At 1.7 mg/kg, all three forms of MDMA, including S(+)-MDMA, significantly increased extracellular serotonin levels. In contrast, at the same dose, only S(+) and S,R(+/-)-MDMA significantly increased extracellular dopamine levels. This effect is consistent with the *in vitro* pharmacological profile of these compounds as S(+)-MDMA has an $EC_{50}$ for the dopamine transporter that is approximately 30 times greater than R(-)-MDMA (Setola et al., 2003). It is also consistent with previous *in vivo* neurochemical measures, as S(+)-MDMA, but not R(-)-MDMA, increases *in vivo* extracellular dopamine turnover in the striatum of rats (Hiramatsu and Cho, 1990; Acquas et al., 2007). These data complement the prolactin results and lend credence to the hypothesis that some additional effect of S(+)-MDMA, presumably dopamine release, functionally antagonizes its effects on prolactin secretion.

One alternative explanation for the different effects of the stereoisomers on dopamine levels in the microdialysis studies could be a difference in potency rather than qualitative differences between the two compounds. Potency differences on this measure are a reasonable expectation because while S(+)-MDMA is 30 times more *potent* than R(-)MDMA at releasing dopamine *in vitro*, R(-)-MDMA does release dopamine under these conditions when the dose is escalated (Setola et al., 2003). Therefore, the effects of R(-)-MDMA were tested at 3.0 mg/kg. At this dose, R(-)-MDMA still did not significantly increase

extracellular dopamine levels in the caudate of these subjects. However, it is still possible that, at higher doses, the R(-) stereoisomer would significantly increase extracellular dopamine levels. This may in fact be likely because, at 3.0 mg/kg, R(-)-MDMA exhibited a greater peak change in extracellular dopamine levels (155%) than it did at 1.7 mg/kg (107%). However, it was decided not to continue increasing the dose of R(-)-MDMA given the heart rate increasing effects of this stereoisomer (Fantegrossi, 2008) and the untoward effects produced by S(+)-MDMA; therefore, this possibility remains untested. Nevertheless, it is clear that at the doses that humans abuse and which rhesus monkeys self-administer, S(+)-MDMA is the only component stereoisomer of MDMA that significantly increases extracellular dopamine levels.

Previous studies have also suggested that there is an *in vivo* interaction between the stereoisomers of MDMA that leads to a “gestalt” racemic mixture with effects that cannot be accounted for by simply summing the individual effects of its component stereoisomers. For example, racemic MDMA elicits a greater locomotor-stimulant effect in mice than either stereoisomer does when administered alone (Fantegrossi et al., 2003). This may be due to facilitation of the dopaminergic effects of S(+)-MDMA, via direct agonism of the 5-$HT_{2A}$ receptor by R(-)-MDMA, as 5-$HT_{2A}$ receptor agonists potentiate dopamine release by racemic MDMA (Gudelsky et al., 1994) and R(-)-MDMA functions as a partial agonist of the 5-$HT_{2A}$ receptor (Nash et al., 1994). To test whether R(-)-MDMA facilitates the dopaminergic effects of S(+)-MDMA in the caudate of rhesus monkeys, a constant amount of S(+)-MDMA was administered in the

presence (1.7 mg/kg S,R(+/-)-MDMA) or absence (0.85mg/kg S(+)-MDMA) of an equal dose of R(-)-MDMA. The results of this experiment show that there is no significant difference between these treatments. This is not supportive of an *in vivo* interaction of these stereoisomers on this measure and further suggests that the dopaminergic effects of racemic MDMA are exclusively mediated by its S(+) stereoisomer.

While the present results indicate that the dopaminergic effects of MDMA are mediated by its S(+) stereoisomer it is important to note the presently reported results were collected in caudate nucleus. Furthermore, there is evidence to suggest that different subregions of the striatum play different roles in the effects of drugs of abuse. In particular, the ventral striatum may play a more pronounced role in the addictive properties of these substances (Haber et al., 2000; Haber et al., 2006; Haber and Knutson, 2010). Despite the possibility of subregional specificity, the caudate nucleus was chosen as the target for these studies due the reliability of surgical targeting of this nucleus, the high level of transporter expression within the caudate, previous results demonstrating that reliable neurochemical measurements can be obtained in nonhuman primates in this nucleus, and the established relationship in this taxa between neurochemical changes in the caudate and the behavioral and reinforcing effects of psychomotor stimulants (Howell and Wilcox, 2001; Howell and Wilcox, 2002; Wilcox et al., 2005; Kimmel et al., 2007; Kimmel et al., 2009). Nevertheless, future studies that compare the neurochemical effects of MDMA and its stereoisomers across different brain regions may further our understanding of the

contribution of different neural systems to the complex biological effects of MDMA. To this end, a brain activational study using fMRI may be of benefit.

Previous work has shown that exposure to MDMA can lead to persistent decreases in prolactin responsivity to acute administration of serotonin releasers such as mCPP and MDMA. Indeed, this “blunting” of the prolactin response has been suggested to be a marker of the integrity of serotonin systems (Hatzidimitriou et al., 2002; Baumann et al., 2008). However, previous studies have also shown that *basal* levels of prolactin are unaltered by exposure to MDMA (Hatzidimitriou et al., 2002). The results of the present study support this finding as we did not observe any significant differences in basal prolactin over time. Furthermore, we found no significant change in extracellular levels of dopamine or its major metabolites, DOPAC and HVA, over the course of the study. However, this study was specifically designed to minimize such effects by scheduling drug administration to occur only once per week, and in randomized order. Further, subjects had previously received significant amounts of both MDMA and cocaine prior to the initiation of these studies. Nevertheless, these results further suggest that exposure to MDMA does not alter basal prolactin levels nor does it alter extracellular levels of dopamine or its metabolites. Whether the response of these substances to drug challenge in rhesus monkeys diminishes with exposure to MDMA remains to be determined.

Additional experiments were undertaken to establish the relationship between the 5-$HT_{2A}$ receptor and MDMA-elicited dopamine release. This receptor may provide a novel target for reducing MDMA abuse as selective

antagonism of this receptor attenuates the locomotor-stimulant, hyperthermic, sleep-disrupting, and reinforcing effects of MDMA (Kehne et al., 1996; Fantegrossi et al., 2002; Fantegrossi et al., 2003; Herin et al., 2005; Chapter 2). These effects are thought to be mediated by central dopaminergic effects (Colado et al., 2004). Further, previous studies suggest that the 5-$HT_{2A}$ receptor is a robust modulator of dopaminergic effects. For example, administration of the 5-$HT_{2A}$ receptor agonist DOI dose-dependently elicits an increase in extracellular dopamine levels in frontal cortex, an effect that is abolished by selective antagonism of the 5-$HT_{2A}$ receptor (Gobert and Millan, 1999). Other work showed that basal dopamine efflux was insensitive to 5-$HT_{2A}$ receptor antagonism (Schmidt et al., 1994; Pehek et al., 2006). Consistent with these findings, in the present work, basal dopamine levels were not significantly altered by administration of the selective (Pehek et al., 2006) 5-$HT_{2A}$ receptor antagonist M100907. This suggests that the modulation of the behavioral effects of MDMA by M100907 is not due to alterations of dopamine tone.

The effects of administration of M100907 on dopamine release were then evaluated. The results of the present study support the hypothesis that the 5-$HT_{2A}$ receptor can modulate dopamine neurotransmission. Consistent with previous findings (Schmidt et al., 1994), these experiments demonstrate that antagonizing this receptor attenuates dopamine release by either amphetamine or MDMA. While recognizing that amphetamine and cocaine are chemically and pharmacologically distinct, this supports previous studies demonstrating that the behavioral effects of the reasonably “pure” psychomotor-stimulants amphetamine

(Chapter 2) or cocaine (McMahon and Cunningham, 2001) can be attenuated by selective antagonism of 5-$HT_{2A}$ receptors. Furthermore, it suggests that the mechanism mediating this behavioral antagonism is a disruption of dopamine release or reuptake inhibition. However, the effects of 5-$HT_{2A}$ receptor antagonism on self-administration of cocaine are somewhat complicated. Antagonism of this receptor attenuates drug (Fletcher et al., 2002) or cue (Nic Dhonnchadha et al., 2009) primed reinstatement of extinguished operant behavior that was previously maintained by cocaine. While controversy exists, this procedure is widely described as modeling drug relapse in humans (Katz and Higgins, 2003). However, across a range of doses, 5-$HT_{2A}$ receptor antagonism was not effective at attenuating ongoing cocaine self-administration (Fletcher et al., 2002; Fantegrossi et al., 2002). Nevertheless, under similar conditions, antagonism of the 5-$HT_{2A}$ receptor attenuated MDMA self-administration (Fantegrossi et al., 2002). While the differential capacity of 5-$HT_{2A}$ receptor antagonism to alter MDMA or cocaine self-administration remains to be determined, it may be related to their distinct pharmacological mechanisms of action. In support of this contention, in these experiments, 5-$HT_{2A}$ receptor antagonism attenuated the behavioral and neurochemical effects of amphetamine, a drug that exhibits a similar pharmacological mechanism to MDMA. In this regard, a study of the effects of 5-$HT_{2A}$ receptor antagonism on amphetamine self-administration would be informative. Furthermore, a study of the effects of 5-$HT_{2A}$ receptor antagonism on cocaine-elicited dopamine overflow would also further our understanding of the relationship between this receptor

and dopamine neurotransmission. Despite these discrepancies, as a whole, this work provides compelling evidence that 5-$HT_{2A}$ receptor antagonism should be continued to be pursued as a novel pharmacotherapeutic target for reducing MDMA abuse and perhaps abuse of other psychomotor-stimulants.

The role of the SERT in the endocrine effects of MDMA was also evaluated. In particular, these experiments focused on establishing whether effects at the SERT mediate MDMA-elicited prolactin secretion. Other work has implicated the SERT in many of the interoceptive and behavioral effects of MDMA, suggesting that direct effects of serotonin mediate these interoceptive and behavioral effects (Gough et al., 1991; Yamamoto et al., 1995; Gudelsky and Nash, 1996; Sabol and Seiden, 1998; Stein and Rink, 1999; Liechti et al., 2000b; Liechti and Vollenweider, 2001; Mechan et al., 2002; Green et al., 2003; Baumann et al., 2007). However, this interpretation would be less tenable if MDMA-elicited secretion of prolactin was also sensitive to SSRI pretreatment. Indeed, It is widely believed serotonin release triggers prolactin secretion (Aloi et al., 1984; Aulakh et al., 1994; Meltzer and Maes, 1995; Baumann et al., 2008). Consistent with this belief, in this study, there was a strong relationship between the timecourse of S,R(+/-)-MDMA-elicited serotonin release and prolactin secretion. In addition, pretreatment with the SSRI fluoxetine attenuated prolactin secretion elicited by administration of S,R(+/-)-MDMA. Furthermore, this effect was surmountable as in the presence of the antagonist S,R(+/-)-MDMA-elicited prolactin secretion at a higher dose. This suggests that fluoxetine attenuated the effects of S,R(+/-)-MDMA on this measure through a competitive interaction at

the SERT. These results indicate that attenuation of the behavioral or interoceptive effects of MDMA may not be sufficient evidence to infer that serotonin itself mediates those effects. In order to dissociate the relative role of prolactin and serotonin in these effects, studies using selective antagonists may be necessary. However, in combination with previous studies (Gudelsky and Nash 1996; Mechan et al., 2002), these data demonstrate that both serotonin release and prolactin secretion elicited by S,R(+/-)-MDMA are attenuated by SSRIs. Regardless of which molecule mediates specific behavioral or interoceptive effects of S,R(+/-)-MDMA, this supports the continued development of SSRIs as pharmacotherpeutics for the treatment of MDMA abuse.

Despite these findings, the effects of fluoxetine pretreatment on R(-)-MDMA-elicited prolactin secretion were more complicated. When administered alone fluoxetine was largely ineffective at attenuating R(-)-MDMA-elicited prolactin secretion. This could have been due to a failure of fluoxetine to attenuate R(-)-MDMA-elicited serotonin release. However, direct microdialysis experiments eliminated this alternative, suggesting that some effect other than serotonin release mediated R(-)-MDMA-elicited prolactin secretion. Previous work has shown that direct 5-$HT_{2A}$ receptor agonists can elicit prolactin secretion (Aulakh et al., 1994) and that R(-)-MDMA can function as a direct agonist of the 5-$HT_{2A}$ receptor both in vitro (Nash et al., 1994) and in vivo (Fantegrossi et al., 2005). Nevertheless, selective antagonism of the 5-$HT_{2A}$ receptor was only partially effective. However, direct agonists at serotonin receptors other than the 5-$HT_{2A}$ receptor can stimulate prolactin secretion (Aulakh et al., 1994). In other

words, it is not a 5-$HT_{2A}$ receptor selective effect. As such, if the serotonin releasing effects of R(-)-MDMA remained intact following treatment with M100907, R(-)-MDMA administration could still elicit prolactin secretion through indirect agonism of serotonin receptors other than the 5-$HT_{2A}$ receptor. This contention was supported by the finding that combined treatment with fluoxetine and M100907 completely attenuated R(-)-MDMA-elicited prolactin secretion. This pattern of effects supports previous findings suggesting a dual mechanism of action of R(-)-MDMA, i.e. serotonin release and direct agonism of the 5-$HT_{2A}$ receptor. Furthermore, this work indicates that combined treatment with an SSRI and a 5-$HT_{2A}$ receptor antagonism may be necessary to attenuate some of the abuse related effects of MDMA.

In summary, the component stereoisomers of MDMA exhibited distinct endocrine and neurochemical effects in rhesus monkeys. Across the dose range tested, these effects were consistent with the hypothesis that S(+)-MDMA functions as a mixed dopamine / serotonin releaser while R(-)-MDMA selectively releases serotonin. A thorough understanding of the complex and distinct effects of the stereoisomers of MDMA will likely enlighten our understanding of the complex pharmacology of racemic MDMA and support therapeutic strategies for the treatment of MDMA abuse. To this end, this work supports previous findings by showing that stereoisomers of MDMA engender qualitatively different effects on the release of dopamine and serotonin and the secretion of prolactin and thereby strengthens the inference that distinct *in vivo* effects of the stereoisomers mediate the complex biological effects of the racemate. Furthermore, this work

indicates that additional research into the effects of the stereoisomers of MDMA is warranted, particularly as it may relate to the complex effects of MDMA in man.

| Hormone / neurochemical | Monkey | First determination | Last determination |
|---|---|---|---|
| **Prolactin (ng/ml)** | **RHp** | 8.57 | 9.30 |
| | **RNb** | 36.70 | 57.90 |
| | **RJt** | 24.99 | 35.82 |
| | **RLt** | 12.77 | 51.21 |
| | **Mean (SEM)** | 20.76 (6.35) | 38.55 (10.79) |
| **Dopamine (nM)** | **RHp** | 11.19 | 6.64 |
| | **RNb** | 8.92 | 6.23 |
| | **RJt** | 3.40 | 4.92 |
| | **RLt** | 1.40 | 2.76 |
| | **Mean (SEM)** | 6.23 (2.29) | 5.13 (0.87) |
| **DOPAC (nM)** | **RHp** | 217.57 | 181.37 |
| | **RNb** | 166.87 | 127.81 |
| | **RJt** | 212.55 | 55.12 |
| | **RLt** | 119.39 | 134.61 |
| | **Mean (SEM)** | 179.10 (22.93) | 124.73 (26.08) |
| **HVA (nM)** | **RHp** | 3503.35 | 3819.53 |
| | **RNb** | 2825.19 | 3562.68 |
| | **RJt** | 3286.21 | 2129.02 |
| | **RLt** | 1927.12 | 2735.76 |
| | **Mean (SEM)** | 2885.47 (349.34) | 3061.75 (387.45) |

**Table 3-1.** Basal levels of prolactin, dopamine, DOPAC, and HVA in rhesus monkeys. First and last determination data are derived from the first and last days of endocrine or dopamine data collection within this study regardless of which treatment was subsequently administered on that particular day.

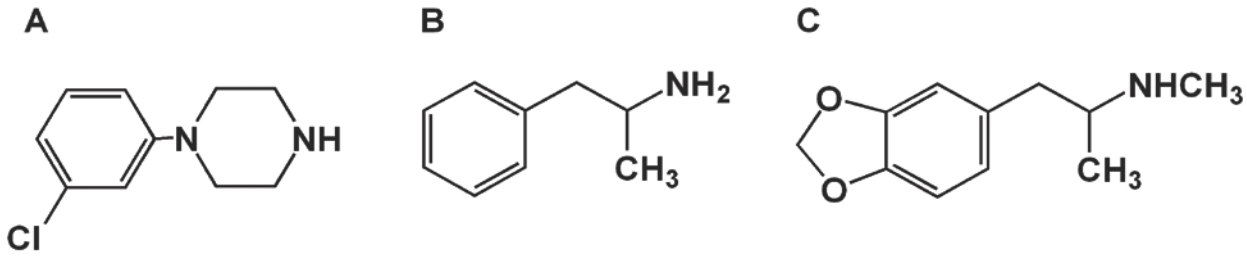


**Figure 3-1.** Chemical structures of test compounds used in this study; including mCPP (A), amphetamine (B), and MDMA (C).

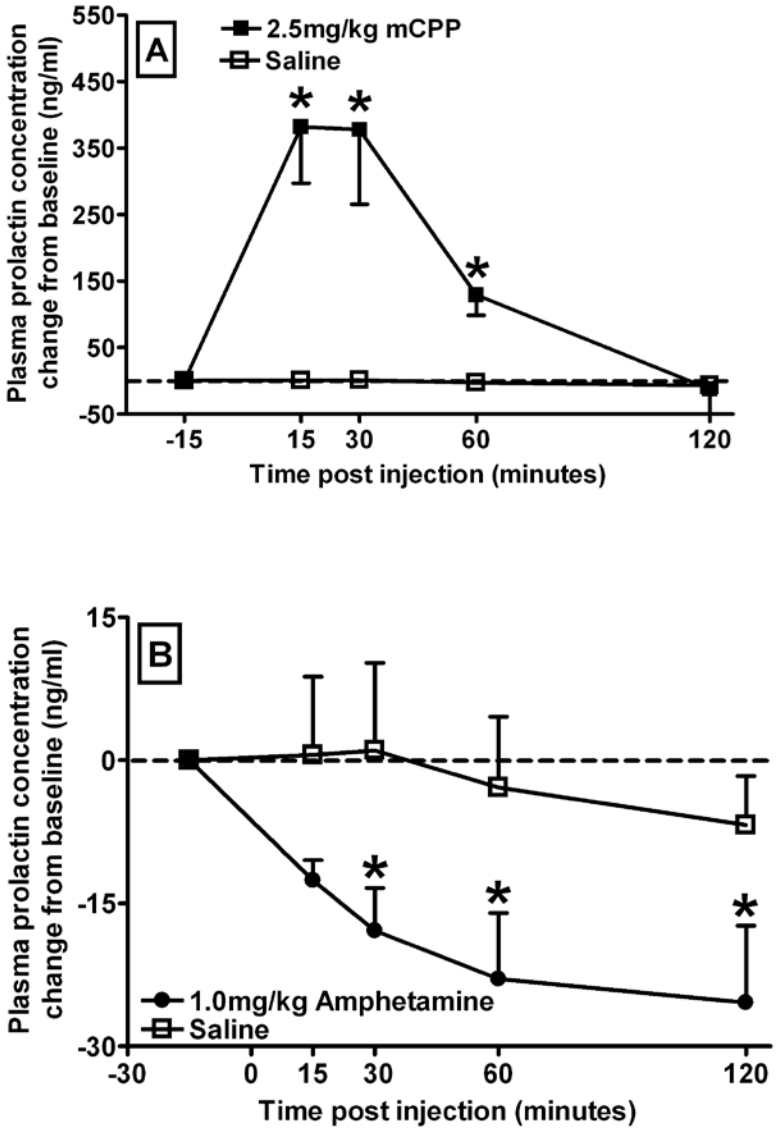


**Figure 3-2.** Effects of the substrate-based serotonin releaser mCPP (2.5 mg/kg, IV; closed squares; A) or the dopamine releaser (+)-amphetamine (1.0 mg/kg, IV; closed circles; B) in comparison to saline (open squares; A and B) on circulating prolactin levels. All points represent the mean ± SEM, and any points without error bars indicate instances in which the SEM is encompassed by the data point. *Abscissae:* Time expressed in minutes in reference to the administration of the test compound and plotted on a linear scale. *Ordinates:* Plasma prolactin concentration expressed as an absolute change from baseline. An * indicates a significant difference from baseline assessed via a one-way repeated measures analysis of variance with post-hoc analysis carried out using Dunnett's test.

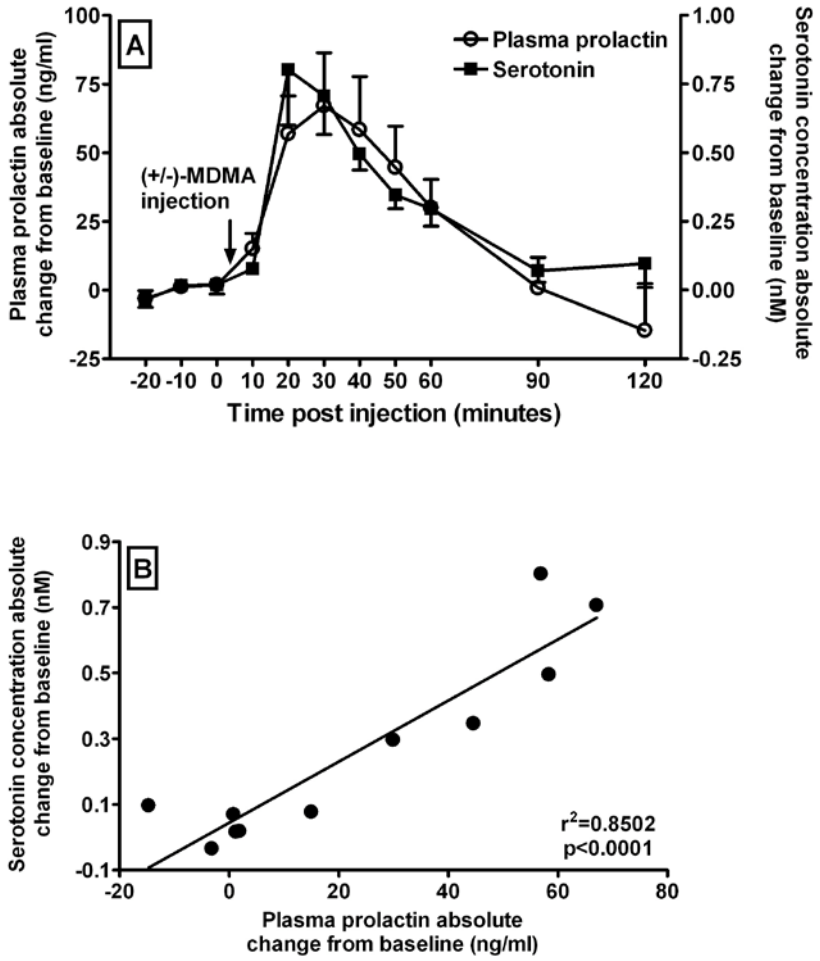


**Figure 3-3.** Timecourse of S,R(+/-)-MDMA (1.7 mg/kg, IV) elicited serotonin release (closed squares; A) and prolactin secretion (open circles; A) and the correlation between each effect (B). *Abscissae:* Time expressed in minutes in reference to the administration of the test compound and plotted on a linear scale (A) or absolute change in plasma prolactin concentration following administration of MDMA (B). *Ordinates:* Absolute change in plasma prolactin concentration following administration of MDMA (A, left) or absolute change in extracellular serotonin concentration following administration of MDMA (A, right and B, left).

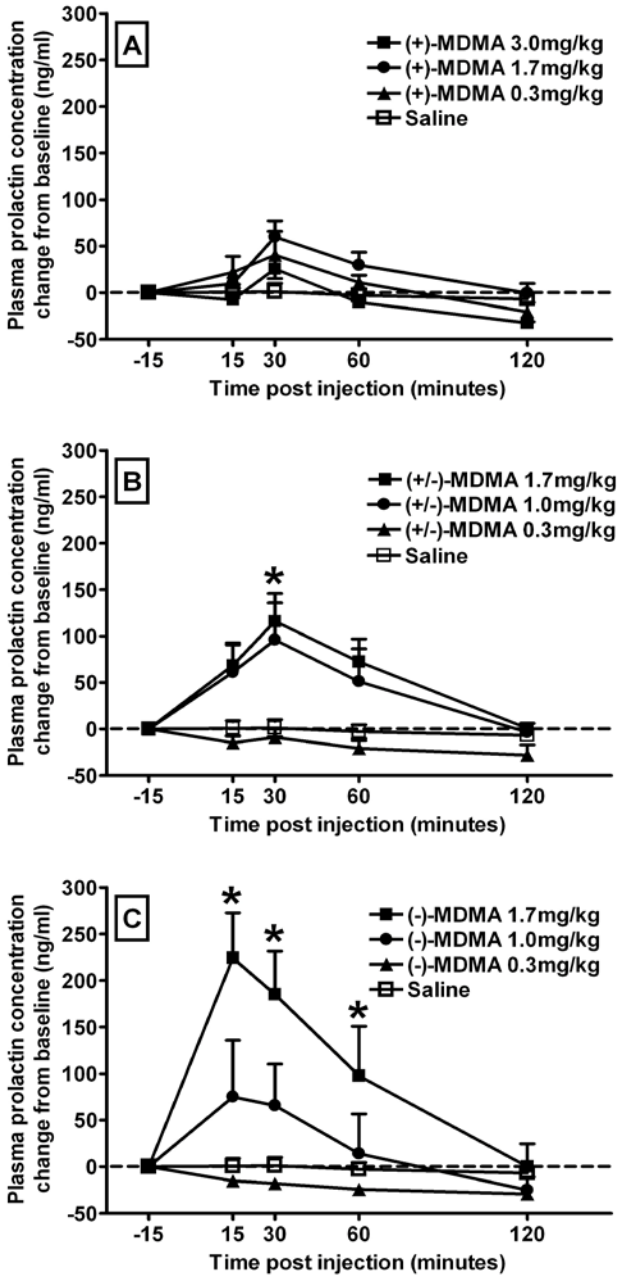


**Figure 3-4.** Dose-effect determination of the effects of S(+)-MDMA (0.3 – 3.0 mg/kg, IV; A), S,R(+/-)-MDMA (0.3 – 1.7 mg/kg, IV; B), and R(-)-MDMA (0.3 – 1.7 mg/kg, IV; C) in comparison to saline (open squares; A, B, and C; redrawn from figure 2) on circulating prolactin levels. The lowest dose administered of a respective form of MDMA is represented by a closed triangle, the intermediate dose by a closed circle, and the highest dose by a closed square. The data at the highest dose of S(+)-MDMA (3.0 mg/kg) are derived from a single subject; in all other cases, the sample size is four. Abscissae, ordinates, and asterisks are the same as in Figure 3-2.

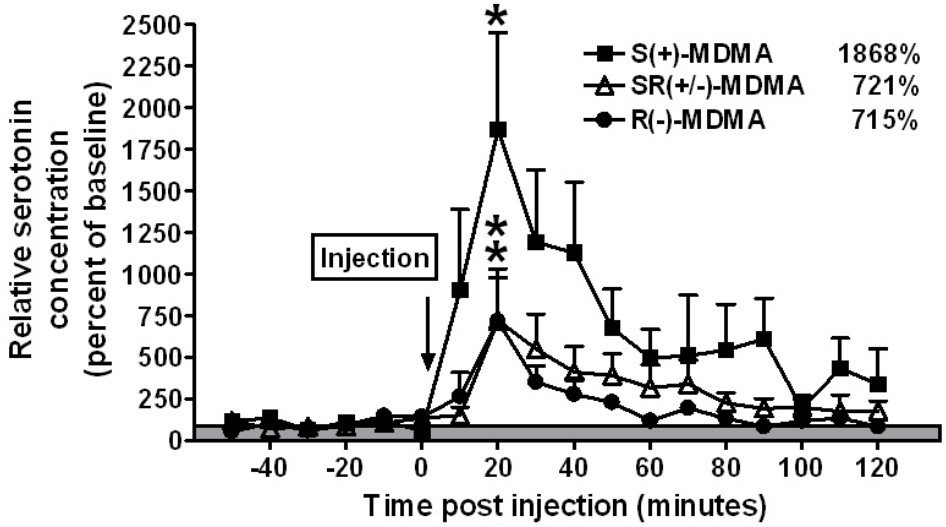


**Figure 3-5.** Effects of S(+)-MDMA (closed square), S,R(+/-)-MDMA (open triangles), and R(-)-MDMA (closed circles) on extracellular serotonin levels within the caudate, at 1.7 mg/kg. This dose of each compound was also found to be the most effective in the prolactin component of this study and is within the range, on a mg/kg basis, that has been reported to be typically taken by human MDMA abusers. The peak effect of each treatment is inset. *Abscissae:* Time expressed in minutes in reference to the administration of the test compound and plotted on a linear scale. *Ordinates:* Extracellular concentration of serotonin within the caudate expressed as a percent change from baseline. An * indicates a significant difference from baseline assessed via a one-way repeated measures analysis of variance with post-hoc analysis carried out via Tukey's test.

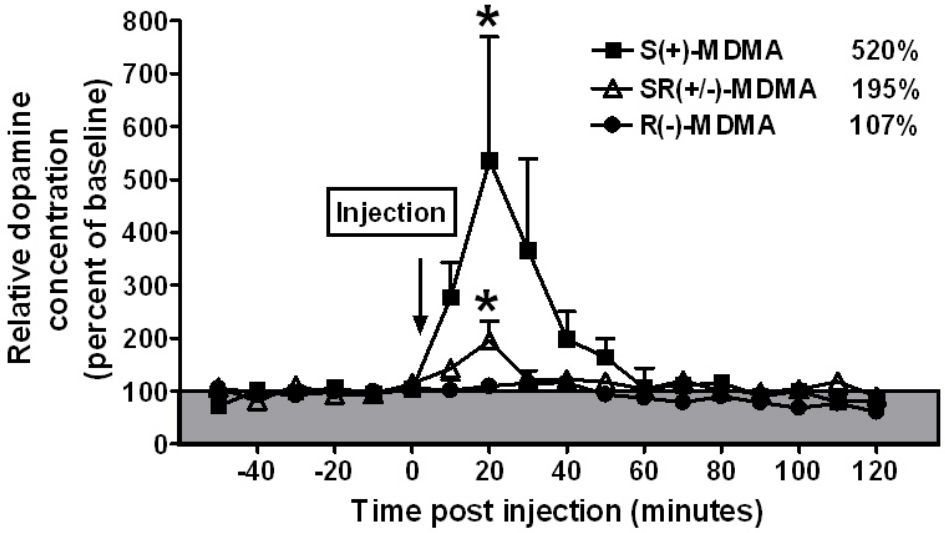


**Figure 3-6.** Effects S(+)-MDMA (closed square), S,R(+/-)-MDMA (open triangles), and R(-)-MDMA (closed circles) at 1.7mg/kg on extracellular dopamine levels within the caudate. The peak effect of each treatment is inset. *Ordinates:* Extracellular concentration of dopamine within the caudate expressed as a percent change from baseline. Abscissae and asterisks are the same as in Figure 3-5.

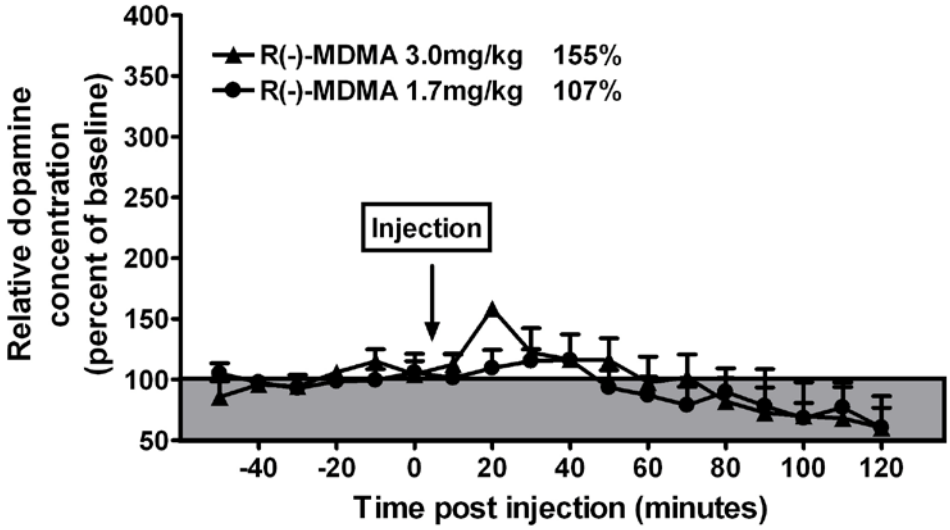


**Figure 3-7.** Lack of effect of R(-)-MDMA at 1.7 mg/kg (closed circles; redrawn from Figure 3-6) or 3.0 mg/kg (closed triangles) on extracellular dopamine levels within the caudate. The peak effect of each treatment is inset. Abscissae, ordinates, and asterisks are the same as in Figure 3-6.

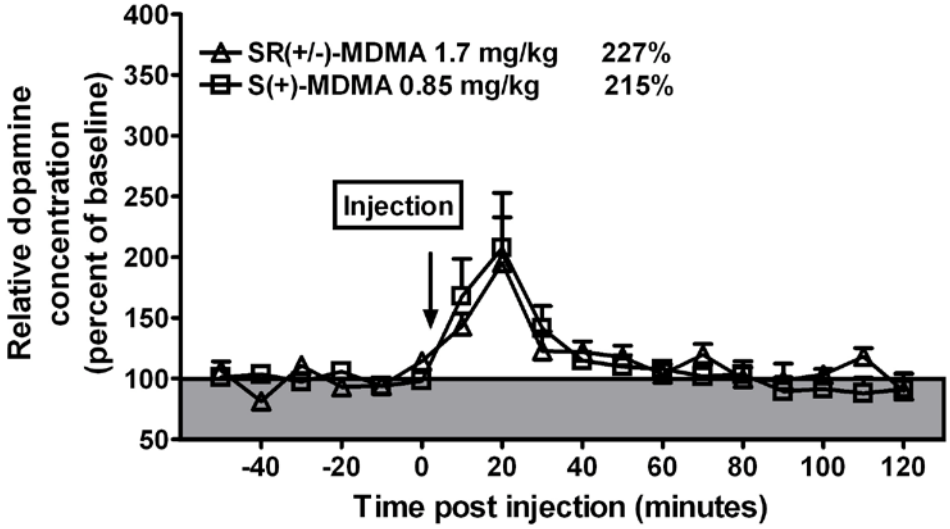


**Figure 3-8.** Comparison of the effect of S(+)-MDMA (0.85 mg/kg, IV; open squares) and S,R(+/-)-MDMA (1.7 mg/kg, IV; open triangles; redrawn from Figure 3-6) on extracellular dopamine levels within the caudate. By halving the dose of S(+)-MDMA administered compared to S,R(+/-)-MDMA, the total amount of S(+)-MDMA administered is equalized between the two conditions as S,R(+/-)-MDMA is composed of equal parts S(+)-MDMA and R(-)-MDMA. The peak effect of each treatment is inset. Abscissae, ordinates, and asterisks are the same as in Figure 3-6.

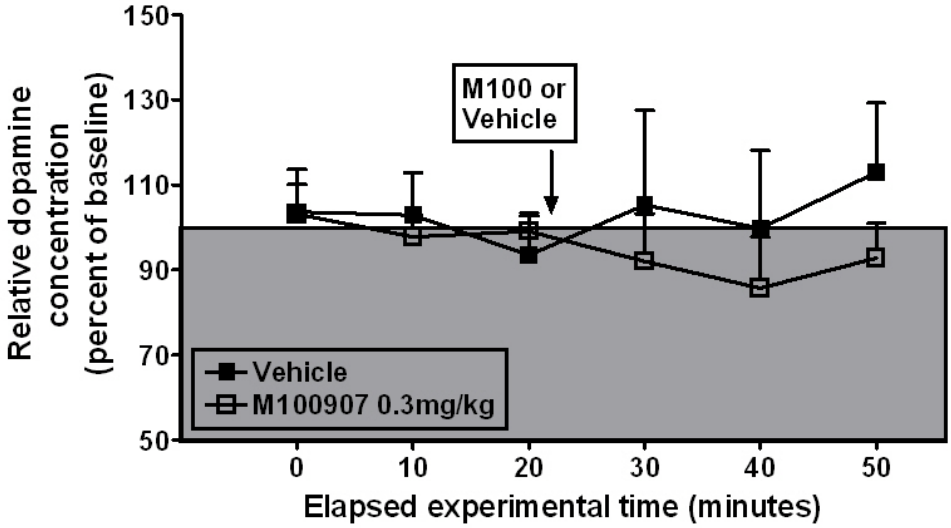


**Figure 3-9.** Determination of the effects of 5-$HT_{2A}$ receptor antagonism by M100907 (0.3 mg/kg, IV) on basal levels of dopamine within the caudate. Abscissae, ordinates, and asterisks are the same as in Figure 3-6.

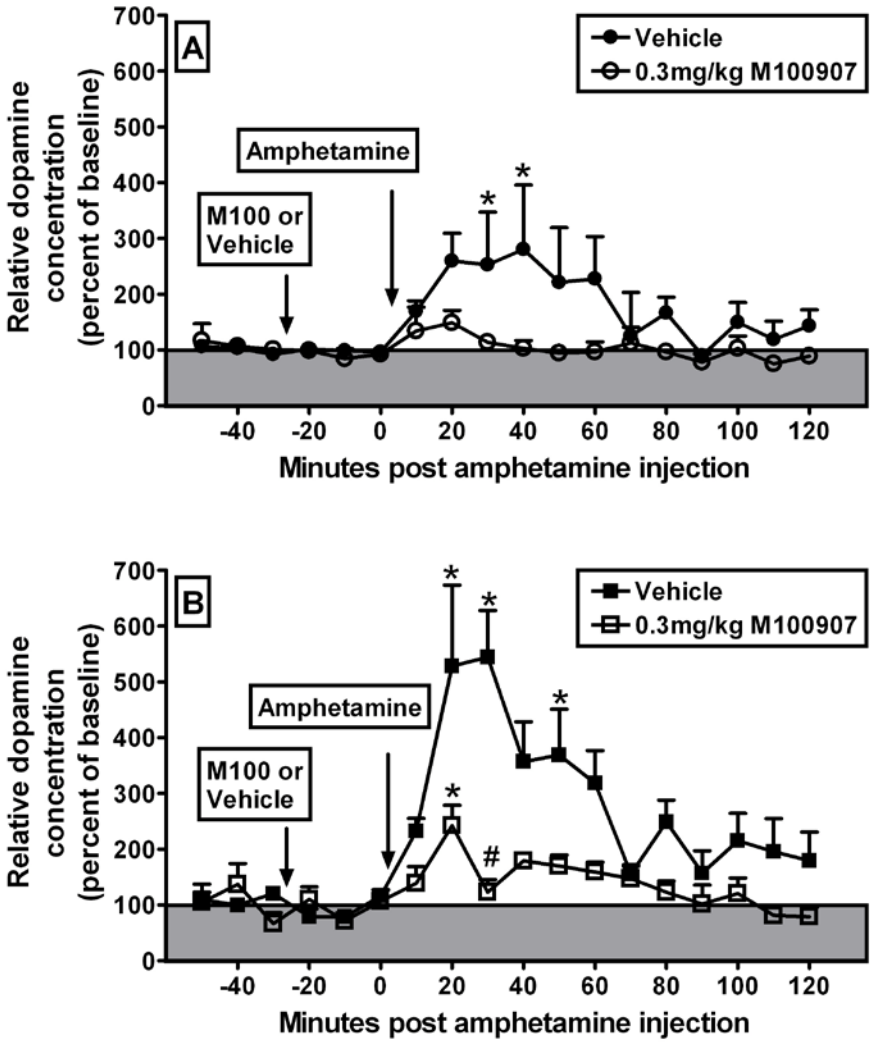


**Figure 3-10.** Determination of the effects of 5-$HT_{2A}$ receptor antagonism by M100907 (0.3 mg/kg, IV) on amphetamine elicited dopamine release within the caudate. Amphetamine was administered intravenously at 0.3 (A) and 1.0 mg/kg (B) following pretreatment with M100907 (open symbols) or its vehicle (closed symbols). Abscissae, ordinates, and asterisks are the same as in Figure 3-6.

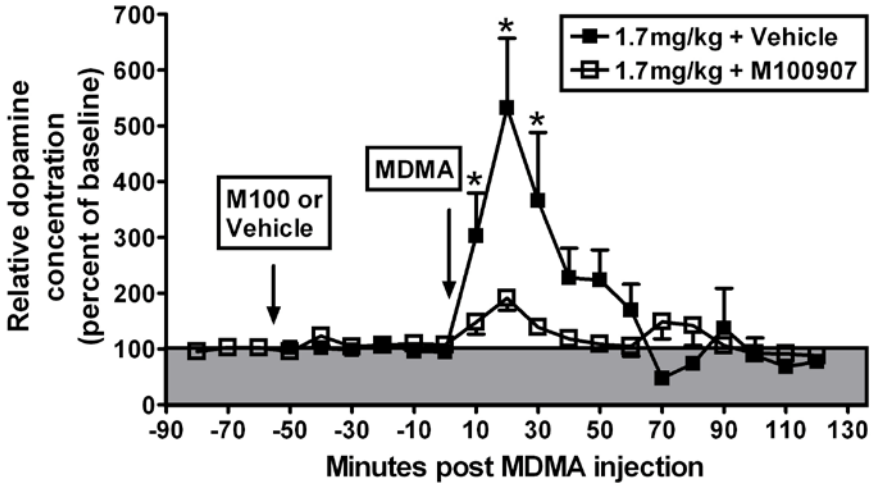


**Figure 3-11.** Determination of the effects of 5-$HT_{2A}$ receptor antagonism by M100907 (0.3 mg/kg, IV) on S(+)-MDMA (1.7 mg/kg, IV) elicited dopamine release within the caudate. Abscissae, ordinates, and asterisks are the same as in Figure 3-6.

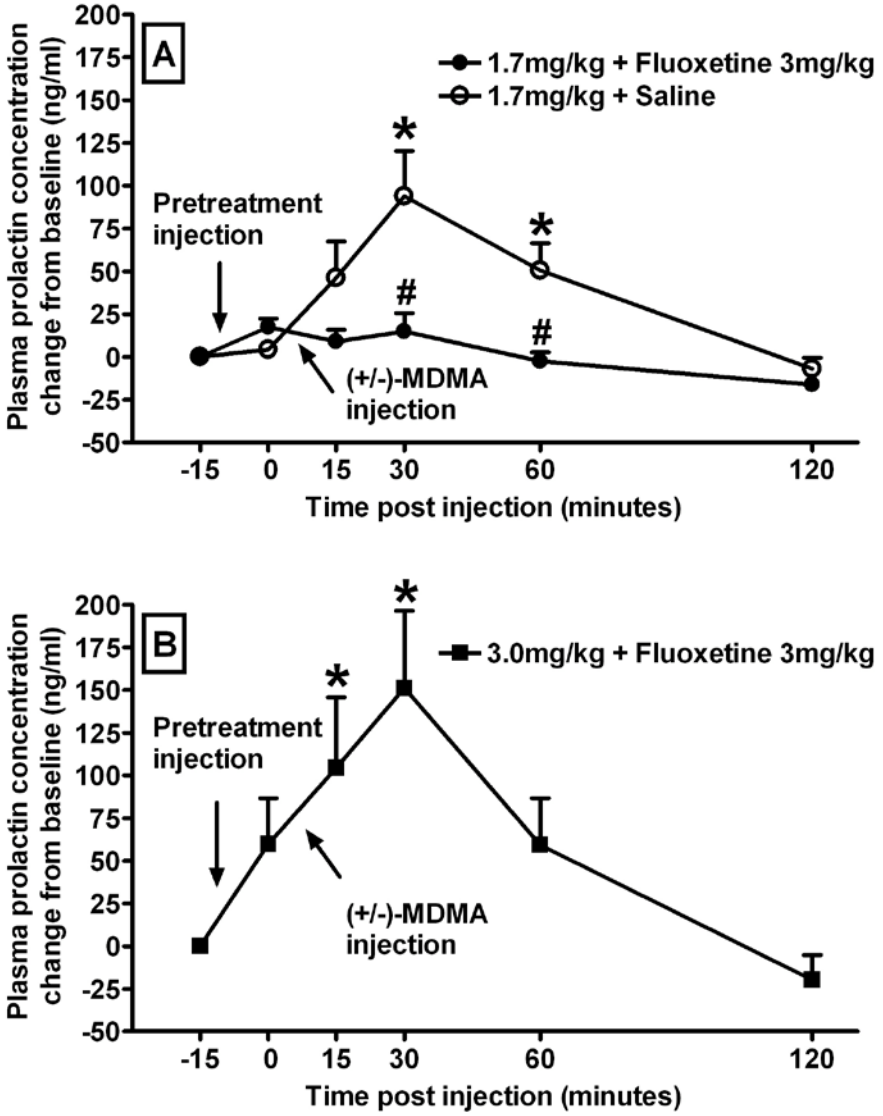


**Figure 3-12.** Determination of the effects of pretreatment with the SSRI fluoxetine (3 mg/kg, IV) on S,R(+/-)-MDMA-elicited prolactin secretion. S,R(+/-)-MDMA was administered intravenously at 1.7 (A) and 3.0 mg/kg (B) following pretreatment with fluoxetine (closed symbols) or saline (open symbols). Abscissae and ordinates, and asterisks are the same as in Figure 3-2. A # indicates a significant difference in prolactin levels at that time point.

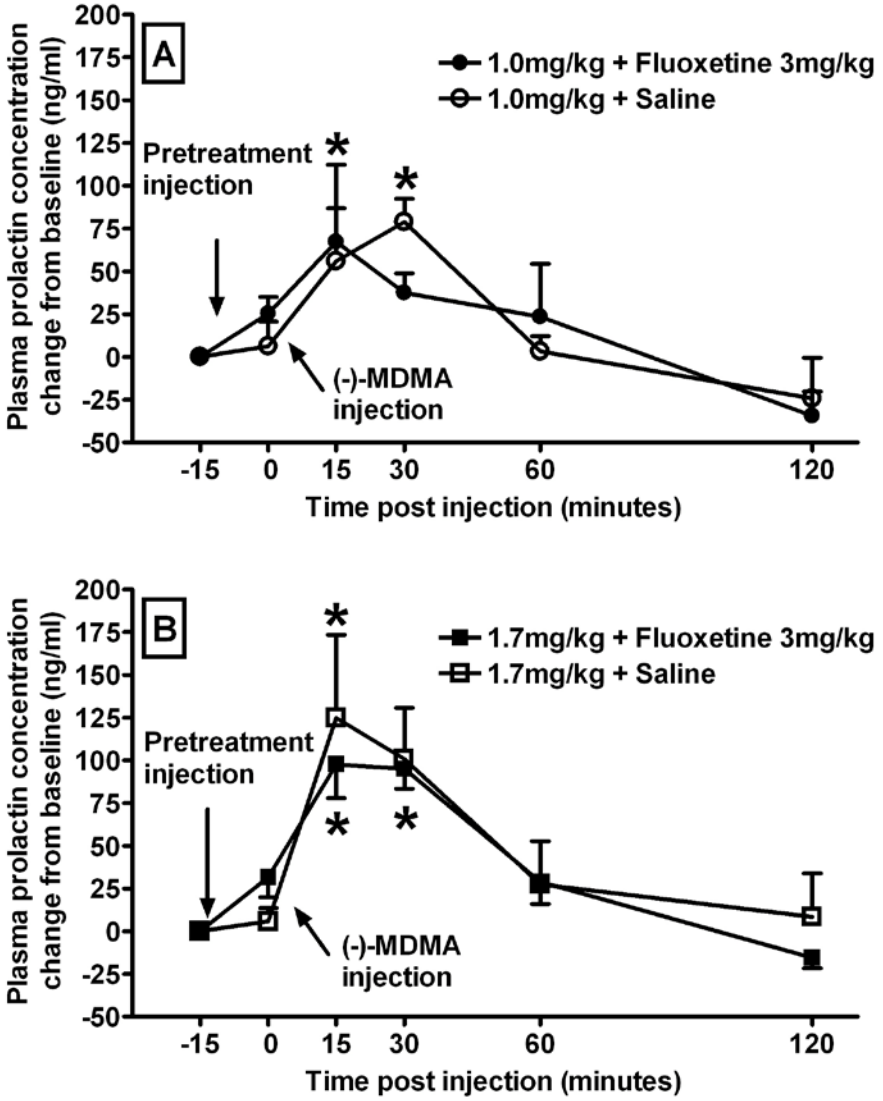


**Figure 3-13.** Determination of the effects of pretreatment with the SSRI fluoxetine (3 mg/kg, IV) on R(-)-MDMA-elicited prolactin secretion. R(+/-)-MDMA was administered intravenously at 1.0 (A) and 1.7 mg/kg (B) following pretreatment with fluoxetine (open symbols) or saline (closed symbols). Abscissae, ordinates, and asterisks are the same as in Figure 3-2.

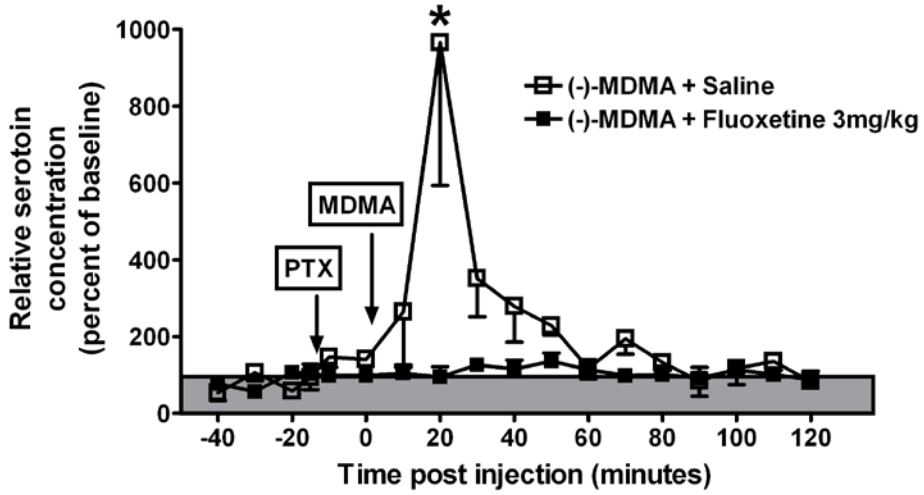


**Figure 3-14.** Determination of the effects of pretreatment with the SSRI fluoxetine (3 mg/kg, IV) on R(-)-MDMA (1.7 mg/kg, IV) elicited serotonin release within the caudate. Abscissae, ordinates, and asterisks are the same as in Figure 3-5.

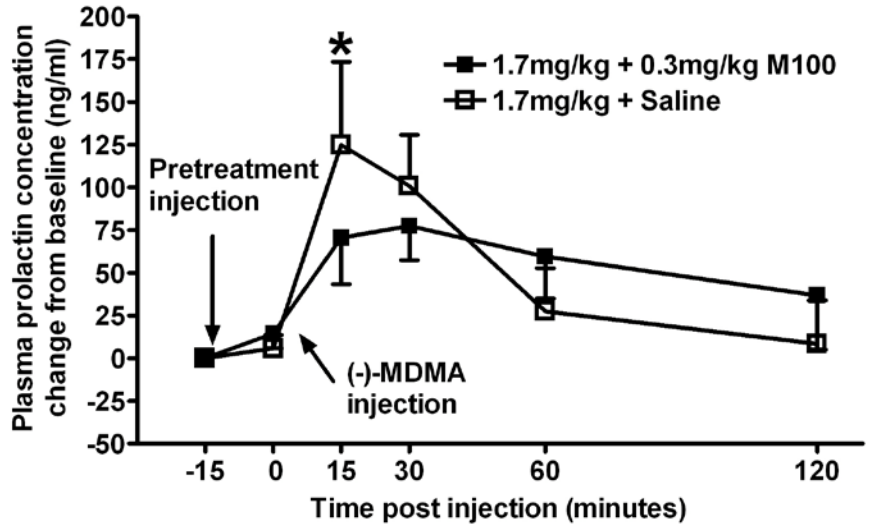


**Figure 3-15.** Determination of the effects of pretreatment with the selective 5-$HT_{2A}$ receptor antagonist M100907 (0.3 mg/kg, IV) on R(-)-MDMA-elicited prolactin secretion. Abscissae, ordinates, and asterisks are the same as in Figure 3-2.

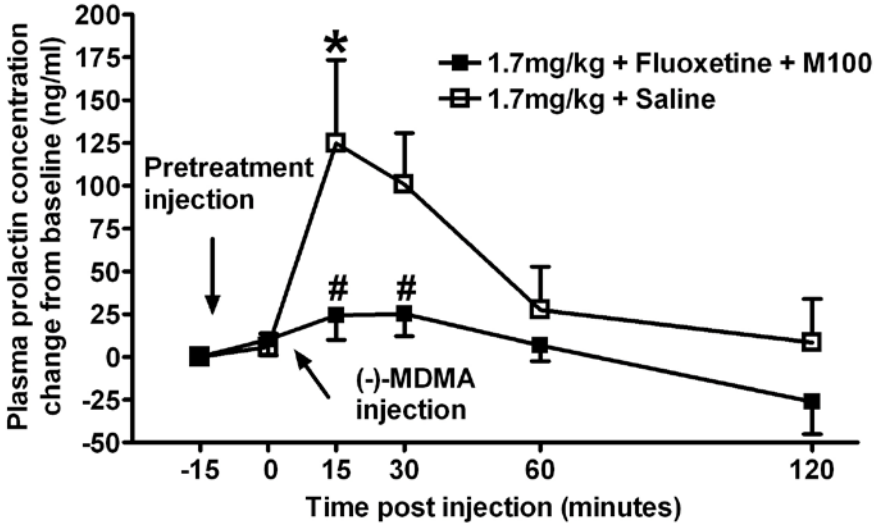


**Figure 3-16.** Determination of the effects of combined pretreatment with the selective 5-$HT_{2A}$ receptor antagonist M100907 (0.3 mg/kg, IV) and the SSRI fluoxetine (3.0 mg/kg, IV) on R(-)-MDMA-elicited prolactin secretion. Abscissae, ordinates, and asterisks are the same as in Figure 3-2.

# Chapter 4: Neuroactivational effects of MDMA

Partially adapted from Murnane KS and Howell LL “Development of an apparatus and methodology for conducting functional magnetic resonance imaging (fMRI) with pharmacological stimuli in conscious rhesus monkeys” Journal of Neuorscience Methods, submitted

## Introduction

Racemic 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) is a substituted phenethylamine with significant abuse liability. MDMA produces complex interoceptive and behavioral effects consistent with a mixture of psychomotor-stimulant and hallucinogen-like effects (Shulgin, 1986; Harris et al., 2002). The development of effective medications for the treatment of MDMA abuse will likely require a thorough understanding of the neuropharmacological underpinnings of these complex effects. To this end, previous studies indicate that the stereoisomers of MDMA engender qualitatively different interoceptive and behavioral effects (see Chapter 2). Importantly, these stereoisomers concomitantly engender qualitatively different neurochemical effects as shown via *in vivo* microdialysis (Chapter 3). These results suggest a parsimonious mechanism for the complex effects of racemic MDMA and indicate that further study of neuopharmacological effects of its stereoisomers is warranted.

*In vivo* microdialysis procedures are powerful as they allow for the direct measurement of extracellular neurochemical levels but are limited in that they provide a discrete sampling of a specific neural locus. Functional neuroimaging provides a powerful complement to these procedures as it allows for whole brain *in vivo* determinations of neuropharmacological effects. As such, there is increasing interest in the use of neuroimaging to study acute pharmacological effects and the development of novel pharmacotherapeutics (Tracey, 2001; Howell and Wilcox, 2002; Wise and Tracey, 2006; Howell and Murnane, 2008). Functional magnetic resonance imaging (fMRI) is a useful modality for these

pursuits as it provides exquisite temporal and spatial resolution while allowing for whole brain coverage. It is important to note, however, that the neurophysiological basis of the signals measured by fMRI are poorly understood (Logothetis, 2002; Logothetis, 2003). Therefore, combined study of pharmacological effects using direct measures and whole brain fMRI provides complementary data with a greater scope than could be provided by either assay in isolation. In the present work, the whole brain neuropharmacological effects of MDMA and its stereoisomers were determined using fMRI.

There are at least four compelling reasons to carry out fMRI research in animals that are not anesthetized. The first is that this facilitates comparisons to human-subject based fMRI experiments that are typically conducted in fully conscious subjects. Second, previous work has shown that anesthesia suppresses the signals measured with fMRI (Brevard et al., 2003). Third, the effects of anesthetics are likely to interact in profound ways with the pharmacological effects of other drugs, such as MDMA, deemed worthy of study. Finally, many important empirical questions require the subjects to actively engage in behavioral tasks. Therefore, in the present work an apparatus and methodology was developed to allow fMRI studies of MDMA and its stereoisomers to be carried out in fully conscious rhesus macaques.

The present study is not the first attempt to develop fMRI techniques applicable to conscious monkeys. However, the approach taken is distinct as these previous studies typically utilized techniques such as the surgical implantation of head posts or pins to minimize motion, the use of reversible

anesthetics to initiate immobilization of the subject, or the use of injected contrast agents to increase the contrast to noise of the functional signal. (Dubowitz et al., 1998; Stefanacci et al., 1998; Ferris et al., 2001; Vanduffel et al., 2001; Andersen et al., 2002; Pinsk et al., 2005; Gamlin et al., 2006; Keliris et al., 2007). While good quality fMRI data can be collected utilizing these procedures, the goal of the present work was to determine if good quality BOLD fMRI data could be collected using a distinct approach. This was deemed worthy of study because head posting requires costly and difficult surgical procedures, can lead to increased susceptibility artifacts, requires maintenance of surgical preparations, and may lead to medical complications that impair the health of the subject. Furthermore, head posting may be most effective when short duration stimuli, such as visual stimuli, are studied as this technique is enhanced by behavioral controls that train the subject to remain still during the stimulus presentation period (Pinsk et al., 2005; Keliris et al., 2007). However, the use of fMRI to study pharmacology necessitates the development of procedures suitable for the sustained timecourse of a pharmacological stimulus (Wise and Tracey, 2006; Howell and Murnane, 2008). Therefore, we sought to develop an apparatus that did not require head posting or initial pharmacological immobilization and thus could be used to study a sustained stimulus with a certainty that residual pharmacological effects were absent. The present work describes the neurobiological effects of MDMA and its stereoisomers, under these conditions.

While compelling reasons exist to acquire fMRI data in conscious subjects, there are also challenges inherent to these types of studies. Either

subject stress or spatial motion can impair the quality of fMRI data. Within this study, extensive efforts were made to minimize any stress to the subjects. The results of these attempts are described as indexed through objective physiological metrics. In addition, subject motion poses a difficult challenge in fMRI research utilizing conscious subjects; particularly when motion is correlated with the presentation of the stimuli (Andersen et al., 2002). The present work describes the results of the pre and post-acquisition techniques utilized to minimize the effects of subject motion.

In addition, positive control experiments were undertaken to validate the neuroanatomical specificity of the data generated using these procedures. Both subject motion and blood oxygenation level dependent (BOLD) fMRI measurements were evaluated by scanning subjects under three different conditions: the absence of stimulation, presentation of a visual stimulus, or administration of i.v. cocaine (0.3 mg/kg). These stimuli have been previously shown to activate specific neural loci (Logothetis, 1999) and as such provide a context for interpreting subsequent determinations of the effects of MDMA. Finally, dose-effect determinations were carried out for MDMA and its stereoisomers. Based on the direct neurochemical effects of MDMA and its stereoisomers, we predicted that S(+)-MDMA would elicit activation of regions innervated by dopaminergic projections, R(-)-MDMA would elicit activation of regions innervated by serotonergic projections, and S,R(+/-)-MDMA would elicit activation of regions innervated by both.

**Methods:**

*Subjects*:

Three adult female rhesus monkeys (*Macaca mulatta*) served as subjects for these studies. All three subjects had a history of exposure to psychoactive compounds and engagement in behavioral experiments. The monkey colony was maintained at an ambient temperature of 22 ± 2°C at 45-50 % humidity, and lights were set to a 12 h light/dark cycle. Each subject was individually housed and fed Purina monkey chow (Ralston Purina, St. Louis, MO), supplemented with fresh fruit and vegetables and water was available *ad libitum* within the colony. Food intake was monitored daily throughout the study by recording the number of chow delivered during each afternoon feeding and the number of chow remaining in the home cage each morning. Any food not consumed by the subject was removed at midnight of the morning preceding an acclimation or data collection procedure. Subjects were closely monitored both during experimental procedures and within the colony for presentation of symptoms consistent with pain or distress. All studies were carried out in accordance with the Guide for Care and Use of Laboratory animals as adopted and promulgated by the National Institutes of Health, and experimental protocols were approved by the Animal Care and Use Committee at Emory University.

*Surgery:*

Each subject was implanted with a chronic indwelling venous catheter into the femoral or jugular vein under sterile surgical conditions as previously

described (Howell and Wilcox, 2001). Catheters were regularly flushed with heparinized saline (100 U/mL) to maintain patency.

*Apparatus:*

In order to acquire quality imaging data without subject motion or stress, specific attention was paid to the development of an apparatus suitable for conducting fMRI studies in conscious rhesus monkeys. The design and implementation of the apparatus was a compromise between rigid head fixation and the maintenance of physiological stability. The frame of this custom apparatus was built out of cylindrical polyvinylchloride (height = 55.9 cm, inner diameter = 30.2 cm, and thickness = 0.6 cm; Figure 4-1, A) and was designed with a detachable front section, a track for a primate collar (length = 15.0 cm, width = 9.5 cm, and height = 1.0 cm) through the top plate (thickness = 3.8 cm) of the frame, and a detachable rear block that allowed it to securely attach to a standard primate chair (Primate Products, Woodside, CA). This design allows subjects to be moved using a standard “pole and collar” technique from their home cage into the restraint cradle without the use of anesthetic immobilization. Subjects could then be transported from the colony to the laboratory or imaging suite. During a procedure, the head was immobilized by a silicone rubber mold (Smooth-Sil 940; Smooth-On, Easton, PA) that was specifically formed for each subject. This mold was created by first forming a near-exact replica of each subject’s head out of plaster as previously described (Howell et al., 2001). The rubber mold was formed to this replica by applying the rubber in a liquid state around the cast and applying a platinum catalyst to transition the rubber to solid

state with a shore hardness of 40A. This hardness was previously determined to provide sufficient head fixation without imposing undue stress to the subject. Figure 4-1, B shows a side profile of an example plaster replica of the head for subjects RBp3. The replica occupies the same space that the head would occupy during a procedure. The replica is partially surrounded by one half of the mold and is positioned as if the subject were looking upwards in the image. Two slits were cut in the mold to allow the subject to ventilate and see visual stimuli. The slit on the right is above the portion of the replica that matches the position of the eyes. The mold was supported on all four sides by acrylic plates that have been formed to fit flush with the side of the mold (Figure 4-1, C). The dorsal (length = 1.1 cm, width = 6.7 cm, and height = 11.7 cm) and lateral side plates (length = 1.1 cm, width = 6.0 cm, and height = 12.4 cm) are flat plates that can be used to add functionality, such as fiducial markers, to the apparatus. Furthermore, these plates contain threaded fiberglass screws that extend through the entire top plate of the apparatus and were used to buttress its structural integrity via the attachment of oversized wing nuts. The ventral plate is curved and fits over the section of the mold that covers the jaw. This piece was used to grossly position the subject in the mold. The subject, surrounding mold, and accessory plates are covered by a (height = 15 cm, inner diameter = 13.3 cm, and thickness = 0.5 cm) polyvinylchloride cylinder designed to fit within the imaging coil (Figure 4-1, D). Body motion poses a particular challenge for fMRI experiments in conscious subjects as previous studies have shown that, even in the absence of head movements, body motion can disrupt the homogeneity of the magnetic field and

produce both image and apparent motion artifacts (Gamlin et al., 2006; Keliris et al., 2007). Here, body motion was reduced by creating an Alpha Cradle IHI/CNR foam insert designed to fill the void in the imaging apparatus not occupied by the body (Smithers Medical Products, Canton, OH). The use of foam inserts has been shown to effectively reduce either head (Howell et al., 2001) or body motion (Gamlin et al., 2006). The insert was reinforced by soft padding and nylon straps that are placed over the torso of the subject. Importantly, the head was maintained closely shaved as this facilitates veterinary examination after each procedure and may serve to keep the subject cooler during the procedure. Furthermore, the scan room was kept cool during imaging sessions and additional cool air was blown over the torso through a series of ventilation tubes (Figure 4-1, E) in an effort to prevent the subject from overheating during the procedure. Subjects were placed within this cradle in the prone position on the bed of the MR scanner (Figure 4-1, F).

*Animal habituation protocol:*

In order to minimize motion and stress, all subjects were extensively and gradually habituated to all procedures necessary for these experiments over a period of several months. Every effort was made to make these protocols routine procedures. The subjects had a previous history of engaging in behavioral experiments and therefore were acclimated to standard primate chairs and “pole and collar” procedures at the initiation of the study. Subjects were first acclimated to transportation within the frame of the custom apparatus, the research staff that carried out this study, and the laboratory that contained a mock fMRI chamber.

Subjects were initially placed in the restraint apparatus and brought to the laboratory for 30 minute sessions three times per week. Over the next month, an increasing number of the pieces of the apparatus were added from session to session until the subject was finally placed in the entire setup for several sessions. Then, the subject was placed into a customized chamber designed to simulate many aspects of the actual scanner (i.e. mock fMRI chamber). Over the next month, the duration of the procedure was gradually increased from 30 minutes to 2 hours and the frequency of immobilization was reduced from 3 times per week to 1 time per week. Over the next several weeks, subjects were acclimated to the noises produced by the MR scanner via audio playback of recordings of several MR pulse sequences (anatomical and functional) at sound levels that were gradually increased to approximate those produced by the MR scanner (up to 110 dB). Importantly, the surrounding head covering provided significant sound attenuation (10 to 20 dB) as this has been shown to be important for fMRI data quality (Andersen et al., 2002). The terminal phase of acclimation involved transportation to the Yerkes imaging center where the subject was immobilized and several sessions were undertaken to expose the subject to the scanner environment prior to the collection of experimental data. Once fully acclimated to the procedure, subjects were habituated to the procedures necessary to obtain physiological measurements. These measurements were subsequently collected over several sessions to objectively evaluate the stress to the subject (see Results). Finally, subjects were acclimated

to administration of i.v. cocaine (0.3 mg/kg) within the apparatus at least 3 times prior to imaging.

*Physiological measurements:*

In order to objectively evaluate whether subjects were experiencing heighted levels of stress, physiological measures were taken when the subjects were in the restraint apparatus and compared to those obtained when the subjects were restrained in a standard primate chair. In each subject, physiological measurements were taken three times over a restraint period of two hours in each condition and averaged (Figure 4-2, A - D). Heart rate data were obtained by securing a pulse oximitry probe to the tail via Vetwrap (3M, St. Paul, MN). Systolic, diastolic, and mean arterial blood pressure data were collected via placement of a non-invasive blood pressure cuff on the right biceps muscle. This cuff was automatically inflated every five minutes during the session. Rectal temperature data were collected via placement of a sheathed and lubricated temperature probe 5 cm into the rectum. Respiratory data were collected via placement of a small bore line close to the nose of each subject. This line was secured by a custom built plastic arm that attached to the lateral side plates and held the line close to the nose of each subject. A potentially problematic system would have to be designed to modify this system for respiratory data collection in a standard primate chair. Therefore, respiratory data were compared in separate sessions when the subject was in the restraint apparatus with or without the custom head mold. All subjects were acclimated to these procedures over several sessions prior to the collection of experimental data. The heart rate and

respiratory rate signals were collected by a SurgiVet V90041 (Smiths Medical, St. Paul, MN) physiological monitor and fed into a desktop PC running AcqKnowledge 3.7.3 (BIOPAC, Santa Barbara, CA) for real-time recording. Blood pressure and temperature data were collected by a SurgiVet V9200 (Smiths Medical, St. Paul, MN) physiological monitor and manually recorded by research personnel every five minutes. Potential stress was also objectively evaluated via measurement of plasma cortisol levels (Figure 4-2, E). All subjects were surgically fitted with chronic indwelling venous catheters (see surgery section). Plasma cortisol levels were determined once in each subject. Baseline levels were taken at the beginning of each procedure by moving the subject to the respective apparatus and immediately transporting the subject to a veterinary procedure room adjacent to the colony. Blood was collected in less than ten minutes. Furthermore, the colony was not disturbed for at least 90 minutes prior to the session. After baseline cortisol level blood sample collection, subjects were immobilized for two hours and blood was collected every 30 minutes. Subjects were habituated to this procedure for several sessions prior to experimental data collection. Samples were assayed by the Yerkes National Primate Research Center's Biomarkers Core Laboratory using a radioimmunoassay as previously described (Sanchez et al., 2005).

*Physiological data analysis:*

Heart rate, respiratory rate, mean arterial blood pressure, and rectal temperature were taken over three sessions whereas endocrine measurements were taken over two sessions. One-way repeated measures analysis of variance

(RM ANOVA) was used to determine if there were significant differences from session to session. Data from each session were then averaged. Subsequently, a two-way RM ANOVA was then used to determine if there were significant differences as a function of the apparatus the subject was in or the time spent in a given apparatus. Post-hoc analysis was carried out via a one-way RM ANOVA with correction for multiple comparisons by the Tukey's test. Graphical presentation of all data depicts mean ± SEM, and any points without error bars indicate instances in which the SEM is encompassed by the data. All graphical data presentations were created using GraphPad Prism 4 (La Jolla, CA), all statistical tests were performed using SigmaStat 3 (San Jose, CA), and significance was arbitrated at a $P<0.05$. All data were binned into 30 minute segments for graphical presentation and data analysis.

*fMRI data acquisition:*

Scans were conducted in a Siemens (Siemens Healthcare, Erlangen, Germany) Trio 3 Tesla magnet with 90cm bore using a (19 cm inner diameter) Siemens CP extremity coil. Anatomical images were acquired using a 3D single shot magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence optimized for T1 contrast. Scan parameters were as follows: TR = 2700ms, TI = 800, 192X192 Matrix, 96mm FOV, 1 NEX, 190 Hz per pixel Bandwidth, 8 degree flip angle, and 9% frequency oversampling yielding a final isotropic resolution of 0.5mm. At least 10 separate collections were averaged off-line for each subject to generate the final anatomical image used for coregistration of the functional data. Anatomical images were acquired in awake

subjects in the imaging apparatus to facilitate coregistration to the functional images. BOLD images were collected utilizing a gradient echo multi-shot echo planar imaging (EPI) sequence; collected after a standard second order shim. These 2D T2*-weighted images were acquired with the following parameters: 47 slices, TR = 4 seconds, TE = 40ms, 64 x 64 data matrix, 96mm FOV, 1594 Hz per pixel Bandwidth, and 90 degree flip angle yielding a final isotropic resolution of 1.5mm. The first 2 images in each time series were discarded to ensure steady state measurements. Furthermore, a saturation pulse was applied during each acquisition to minimize extraneous effects at the boundary of the cranium. Finally, field inhomogeneities were mapped using a standard Siemens phase and magnitude image collection sequence for later correction of any EPI image distortions (Figure 4-3). These scan parameters provide a balance between signal, contrast, resolution, and facilitate co-registration to functional data. In particular, a multishot EPI sequence was used to minimize dynamic and off-resonance image distortions. Relatively small and isotropic voxels were used to minimize the effects of motion and to facilitate coregistration to anatomical images, respectively. Finally, a 40 ms TE was found to enhance the sensitivity of BOLD signal measurements without resulting in excessive signal drop-off.

*Baseline motion:*

Each subject underwent three different fMRI acquisitions. To evaluate baseline motion, a scan was obtained in the absence of any stimulation. This scan was composed of 100 image acquisitions and lasted 6.67 minutes (100 acquisitions X 4 second TR = 400 seconds).

*Visual stimulation:*

Subjects were presented with an alternating checkerboard visual stimulus designed to elicit activation of primary visual cortex (Logothetis, 1999). This stimulus was composed of alternating full contrast black and white squares. Stimuli were presented in four blocks composed of a 60 second epoch without stimulation, followed by a 30 second epoch of stimulus presentation, and terminating with an additional 60 second epoch without stimulation. The stimulus was alternated at 5 Hz and occupied 15 degrees of the visual field. Stimuli were presented using a Pentium III workstation under timing control by Presentation software (Neurobehavioral Systems, Albany, CA), at a resolution of 640 X 480 pixels, and at a frame rate of 60 Hz.

*Drug administration:*

The within-session experimental timeline when either cocaine or MDMA were administered began with baseline data collection for two minutes, followed by a saline infusion, followed by 2 minutes of additional scanning, followed by an intravenous infusion of drug, and finally 6 minutes of subsequent scanning. This paradigm allowed for a within-session negative control (i.e. saline infusion) and was based on previous data showing a robust increase in blood flow or blood oxygenation within 5 minutes of a bolus of cocaine (Howell et al., 2001; Howell et al., 2002; Howell et al., 2009) or MDMA (Brevard et al., 2006). Cocaine, S,R(+/-)-MDMA, S(+)-MDMA, and R(-)-MDMA were supplied by the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC) and

dissolved in 0.9% saline. Throughout this study the infusion rate and volume were held constant at 15 ml/min and 4ml, respectively. The dose of either drug is expressed as the salt form. Cocaine was administered intravenously at 0.3 mg/kg. Subsequent intravenous administrations were carried out with S,R(+/-)-MDMA (0.3 and 1.0 mg/kg), S(+)-MDMA (0.3 mg/kg), and R(-)-MDMA (0.3 and 1.0 mg/kg). These doses were chosen as a balance between previously observed acute neurophamacological effects and safety concerns with particular relevance when subjects were administered drugs under conditions of restraint.

*Spatial motion analysis:*

Translation and rotation data were determined during each of the three scans and analyzed separately. The maximum translation and rotation from one acquisition to the next across the entire time series and across all three scans was compared to specific criteria (translations to one half the size of the voxel size or 0.75 mm and rotations to 1.5 degrees) via a one sample t-test. Furthermore, two-way RM ANOVA was utilized to compare the maximum, mean, and the variability of translational and rotational motion across axis and scan condition. For these analyses realignment parameters were transformed by taking the absolute value of the difference from one acquisition to the next and therefore represent absolute motion across acquisitions. Graphical presentation of all data depicts mean ± SEM, and any points without error bars indicate instances in which the SEM is encompassed by the data. All graphical data presentations were created using GraphPad Prism 4 (La Jolla, CA), all statistical

tests were performed using SigmaStat 3 (San Jose, CA), and significance was arbitrated at a $P<0.05$.

*fMRI data analysis:*

Analyses were carried out using the standard image analysis package Statistical Parametric Mapping version 5 (SPM5 – Wellcome Trust Center for Neuroimaging, London, UK) supplemented by custom software written in the matrix based programming environments IDL (ITT, Boulder, CO) and MATLAB (MathWorks, Natick, MA). Preprocessing of the images was initiated via placement of both the anatomical and functional images in AC-PC alignment and in gross registration to one another. Time series realignment using a 6 parameter rigid body algorithm (Woods et al., 1993; Cox and Jesmanowicz, 1999) to reduce the influence of any subject motion was then carried out. Concurrently, field inhomongeneity data were used to correct any geometric distortions in the EPI images using an automated algorithm that takes into account the interaction between motion and inhomogeneities and has been shown to result in an improved coregistration between EPI and T1 images (Cox and Jesmanowicz, 1999; Hutton et al., 2002). Anatomical data were then segmented into gray matter, white matter, and bias corrected images. Functional data were then spatially normalized to the bias corrected anatomical images and spatially smoothed using a kernel with a full width at half max equal to two times the native resolution of the image (i.e. 3mm). Linear drift was accounted for by global normalization across the time series and high-pass filtering. Whole brain analysis was carried out on a pixel by pixel basis using a parametric general linear

statistical model. This analysis was confined to gray matter pixels. Motion parameters were used as covariates within this model to remove the influence of subject motion on the subsequent results. Individual subject analyses were conducted using a general linear model fit that was based on a flexible boxcar design with corrections for multiple comparisons such that the probability of a type I error was maintained at 5% (Genovese et al., 2002). Group analysis was conducted using a one-sample t-test of the statistical values generated by the individual subject analyses.

**Results:**

Under the conditions employed, rhesus monkeys could be reliably acclimated to undergo fMRI scans while awake. The integrity of the imaging data necessitated that subjects were minimally stressed and near motionless. To objectively assess the effectiveness of the training procedure in minimizing any stress to the subject, physiological and endocrine measurements were taken in fully acclimated subjects over two hour sessions in either the custom fMRI apparatus or in a standard primate chair (with the exception of the respiratory rate data – see Methods). In each condition, physiological measurements were taken over three sessions whereas endocrine measurements were taken over two sessions. In the custom fMRI cradle, one-way RM ANOVA reveal no main effect of heart rate ($F_{2,2} = 0.295$; $p = 0.760$), respiratory rate ($F_{2,2} = 2.027$; $p = 0.212$), blood pressure ($F_{2,2} = 0.051$; $p = 0.951$), and temperature ($F_{2,2} = 5.528$; p

= 0.096) as a function of session. In the primate chair (or custom apparatus without head restraint for respiratory rate data), heart rate ($F_{2,2}$ = 2.537; p = 0.194), respiratory rate ($F_{2,2}$ = 2.501; p = 0.125), blood pressure ($F_{2,2}$ = 2.154; p = 0.213), and temperature ($F_{2,2}$ = 0.967; p = 0.454) were not significantly different as a function of session. Data from each session were then averaged. A two-way RM ANOVA was then used to determine if there were significant differences as a function of the apparatus used or the time spent in a given apparatus. Heart rate ($F_{2,1}$ = 0.074; p = 0.811), respiratory rate ($F_{2,1}$ = 0.342; p = 0.618), blood pressure ($F_{2,1}$ = 1.875; p = 0.304), rectal temperature ($F_{2,1}$ = 0.002; p = 0.968), and plasma cortisol levels ($F_{2,1}$ = 23.095; p = 0.131) did not significantly differ by condition. Furthermore, there was no main effect of time spent in the apparatus for heart rate ($F_{2,3}$ = 0.395; p = 0.762), respiratory rate ($F_{2,3}$ = 3.156; p = 0.107), blood pressure ($F_{2,3}$ = 1.152; p = 0.402), or rectal temperature ($F_{2,3}$ = 0.402; p = 0.757). In contrast, there was a significant main effect of time spent in each apparatus on plasma cortisol levels ($F_{2,5}$ = 7.978; p = 0.020). Post-hoc analysis via Tukey's test revealed that in the custom cradle the baseline time point was significantly different from all of the subsequent time points ($p < 0.050$) but none of the subsequent time points was significantly different from the others. This same analysis showed no main effect of time in the standard primate chair ($F_{2,5}$ = 4.929; p = 0.052). While a significant effect of time was measured in the custom apparatus but not in the standard primate chair, no significant main effect of condition was measured.

In addition to a stable physiology, good quality fMRI data requires minimal subject motion. Figure 4-4 shows transformed realignment parameters across the three translational and rotational axes, assuming rigid body motion, averaged across the three subjects. These data are summarized in Table 1 as expressed by the maximum, mean, and standard deviation of the motion from acquisition to acquisition in each axis. One-sample t-test revealed that translational and rotational movements were significantly less ($p < 0.05$) than criterion for all axes and conditions except Z-axis translations ($t_3 = -3.066$; $p = 0.092$) and X-axis rotations ($t_3 = -1.537$; $p = 0.264$) during visual stimulation. Two-way RM ANOVA revealed that, for the maximum translational motion from scan to scan, there was no main effect of axis (X, Y, Z ; $F_{2,2} = 2.500$; $p = 0.197$) or condition (No stimulation, visual stimulation, cocaine; $F_{2,2} = 2.257$; $p = 0.221$) and no significant interaction ($F_{2,4} = 0.901$; $p = 0.507$). Furthermore, there was no main effect of axis ($F_{2,2} = 0.156$; $p = 0.860$) or condition ($F_{2,2} = 1.894$; $p = 0.264$) and no significant interaction ($F_{2,4} = 0.476$; $p = 0.753$) for maximum rotations. Mean translational motion from acquisition to acquisition showed a main effect of axis ($F_{2,2} = 7.623$; $p = 0.043$) but not condition ($F_{2,2} = 2.462$; $p = 0.201$) and there was no significant interaction between these factors ($F_{2,4} = 1.473$; $p = 0.297$). However, post hoc analysis via the Tukey's test did not show any significant individual differences ($p < 0.050$) between the three axes. Moreover, mean rotational motion showed no main effect of axis ($F_{2,2} = 1.220$; $p = 0.386$), condition ($F_{2,2} = 0.582$; $p = 0.600$), and no significant interaction ($F_{2,4} = 1.936$; $p = 0.198$). The variability (standard deviation) of translational motion also did not

vary as a function of axis ($F_{2,2}$ = 4.762; p = 0.087) or condition ($F_{2,2}$ = 3.051; p = 0.157) and there was no significant interactions between these factors ($F_{2,4}$ = 1.233; p = 0.370). Finally, the variability of rotational motion did not show a main effect of axis ($F_{2,2}$ = 0.900; p = 0.476), condition ($F_{2,2}$ = 2.189; p = 0.228), and no significant interaction ($F_{2,4}$ = 0.654; p = 0.641).

Functional activity was assessed via BOLD fMRI signal changes following visual stimulation or acute cocaine challenge in all three subjects. Presentation of the visual stimulus elicited activation that was principally localized to the primary visual cortex (Figure 4-5). These results remained after correction for multiple comparisons and were consistent across the three subjects. Furthermore, cocaine challenge elicited activation that was principally localized to the anterior cingulate and the dorsal regions of the prefrontal cortex – specifically the medial dorsal regions - (Figure 4-6). While there was some individual variability in the response to cocaine, these results also survived correction for multiple comparisons and were consistent across the three subjects. Furthermore, cocaine administration sporadically activated temporal and parietal regions of the cerebrum.

Finally, the neuroactivational effects of S,R(+/-)-MDMA, S(+)-MDMA, and R(-)-MDMA were assessed under the same conditions used to determine the effects of cocaine. Similarly to cocaine, S,R(+/-)-MDMA significantly increase blood oxygenation in the anterior cingulate and the medial / dorsal regions of prefrontal cortex (Figure 4-7). In contrast, however, S,R(+/-)-MDMA engendered increased blood oxygenation in many regions insensitive to the effects of

cocaine. These regions include the striatum, primary visual cortex (V1), primary auditory cortex (A1), posterior cingulate, amygdala, thalamus, hippocampus, and hypothalamus. These cortical regions activated by S,R(+/-)-MDMA can be largely categorized to include portions of the frontal, temporal, and parietal lobes and receive both dopaminergic and serotonergic innervations (Figure 4-8) whereas cocaine selectively elicits cortical activation in the frontal lobe regions that predominantly receive dopaminergic innervation(Figure 4-9). The effects of S(+)-MDMA were remarkably similar to the effects of S,R(+/-)-MDMA (Figure 4-10) whereas the effects of R(-)-MDMA were selective for regions in temporal and parietal cortex that predominatly receive serotonergic innervations with very little activation of frontal cortex (Figure 4-11).

**Discussion:**

The present study documents the utility of the described apparatus and procedures for the conduct of fMRI experiments in conscious nonhuman primates. An effective restraint device was developed that facilitated immobilization by readily attaching to a standard primate chair. Specific attention was paid to developing an apparatus that did not require the subjects to be surgically fitted with head posts. This reduced the invasiveness of the procedure, the need for a surgical preparation that may be associated with medical complications, and the possibility of surgical preparation-related susceptibility artifacts. Furthermore, this device allowed for the immobilization of the subject

without initial anesthetic induction. Therefore, subjects could be scanned in the absence of residual anesthetic effects that may represent a significant confound, particularly in the context of pharmacological imaging.

High quality fMRI data requires stable physiology and minimal spatial motion. The effectiveness of the acclimation procedure in allowing subjects to meet these requirements was assessed via objective metrics. Direct comparisons were made for physiological parameters during immobilization in the restraint apparatus to immobilization in a standard primate chair. Heart rate, mean arterial blood pressure, rectal temperature, and plasma cortisol levels did not differ between these conditions. Furthermore, respiratory rate was unaffected by encasement of the entire head in an individually fitted mold. These data indicate that these subjects were not more stressed by these custom procedures than by standard primate immobilization procedures. Spatial motion was assessed by determining the absolute translational and rotational motion during the three different scan conditions. Imaging data were acquired in the absence of stimulation, during presentation of a visual stimulus, and both before and after intravenous administration of cocaine (0.3 mg/kg). Spatial motion did not significantly differ as a function of these conditions. Furthermore, motion did not significantly differ along the three spatial axes. Mean translation motion across the three conditions and the three axes varied from 0.009 to 0.07 mm whereas mean rotational motion varied from 0.07 to 0.20 degrees. Mean translation motion across the three conditions and the three axes varied from 0.05 to 0.33 mm whereas mean rotational motion varied from 0.39 to 0.91 degrees.

Moreover, over the course of the three scans, the maximum translational and rotational motion, from acquisition to acquisition, rarely exceeded one half of the voxel size (0.75 mm) nor 1.5 degrees, respectively. These data indicate that the subjects remained relatively motionless during these procedures. These results clearly document that utilizing these procedures rhesus monkeys can be reliably acclimated to undergo fMRI experiments while awake and with minimal stress or motion.

High quality functional (EPI) and anatomical (T1W) images were also reliably acquired with these procedures. These images showed good signal to noise and relatively little artifact from motion or susceptibility differences. However, EPI image acquisition techniques are inherently sensitive to field inhomogeneities and thereby produce geometric distortions in the resultant image. Therefore, a field mapping technique was used to determine the vectors of the magnetic field inhomogeneities. The EPI images were then corrected by applying the inverse of the calculated field map. These procedures resulted in images that were a closer spatial approximation of the anatomy of an individual subject and have been shown to improve coregistration of the two image types (Jezzard and Balaban, 1995; Hutton et al., 2002). In addition, the anatomical specificity of stimulus-induced neural activation was verified by presenting the subjects with a visual stimulus. Similar stimuli have been shown to principally increase blood oxygenation in primary visual cortex (Logothetis et al., 1999). The results of this study corroborate these findings by showing that the areas of significant blood oxygenation changes were largely localized to primary visual

cortex. Taken together, these data show that under these procedures good quality images could be obtained and validate the anatomical specificity of the measured changes in blood oxygenation.

Administration of 0.3 mg/kg of cocaine resulted in a significant increase in blood oxygenation of the anterior cingulate and the medial / dorsal regions of prefrontal cortex. This effect is consistent with cocaine-induced changes in cerebral blood flow (CBF) in unanaesthetized rhesus monkeys (Howell et al., 2002; Howell and Murnane, 2008; Howell et al., 2009). Furthermore, the cerebral metabolic changes engendered by cocaine in unanesthetized rhesus monkeys also showed localization to the dorsal regions of the prefrontal cortex (Henry-Kirkland et al., in press). The concordance of these data sets further supports the anatomical specificity of the changes in blood oxygenation reported in the present study. This brain activation pattern of cocaine may be different from the patterns produced by related psychomotor-stimulant drugs of abuse such as amphetamine and methylenedioxymethamphetamine (MDMA). If important differences exist in the brain activation patterns produced by different drugs of abuse it may have important implications for understanding the behavioral and interoceptive effects of these drugs and for the development of medications that attenuate their addictive properties.

Previous data suggests that amphetamine appears to produce a more widespread activation of regions innervated by dopaminergic terminals than cocaine (Jenkins et al., 2004; Schwarz et al., 2007). The more pronounced effects of amphetamine than cocaine on dopaminergic neuroactivation is

consistent with direct neurochemical measurements demonstrating that, across a range of doses, amphetamine increases extracellular dopamine levels to a greater extent than does cocaine (Kimmel et al., 2007; Kimmel et al., 2009). Further study suggests that the neuroactivational effects of MDMA have a serotonergic component that may be lacking in the response to either amphetamine or cocaine (Brevard et al., 2006; Meyer et al., 2006). However, these studies were carried out with significant methodological differences. In the present study, the neuroactivational effects of cocaine, MDMA, and the stereoisomers of MDMA were determined within subject and within modality. This allowed for a controlled comparison between drugs.

As previously described, administration of cocaine elicited activation of the anterior cingulate and the medial / dorsal regions of prefrontal cortex. These regions receive extensive and relatively selective dopaminergic input (Cooper et al., 2003; Kandel et al., 2000). This finding is consistent with the prominent role of dopamine in the neuropharmacology of cocaine and other psychomotor-stimulants (Ritz et al., 1989; Cabib et al., 1991; Wilcox et al., 2002). S,R(+/-)-MDMA dose-dependently elicited activation of these same brain regions. Indeed, S,R(+/-)-MDMA-elicited activation of the striatum, a region that also receives relatively selective dopaminergic innervations and has been tightly linked to the behavioral and interoceptive effects of psychomotor-stimulants (Haber 1986; Haber and Fudge 1997; Haber and Knutson 2010). The dose-dependent nature of this BOLD response to MDMA is an important validation of this technique for the study of pharmacology. *In vivo* dose-dependence is an important, albeit

indirect, index that the effect measured depends on the concentration of the drug at its site of action, a central tenet of pharmacology. Previous fMRI studies sensitive to changes in CBV had some success in measuring a dose-dependent relationship with bicuculline (Reese et al., 2000) and cocaine (Marota et al., 2000). Other results demonstrated that cerebral blood flow (CBF) is also dose-dependent, as measured by $O^{15}$ labeled water via PET (Howell et al, 2002). While the BOLD, CBV, and CBF responses are correlated (Logothetis, 2003; Nair, 2005), there have been limited and contradictory reports of BOLD dose-dependency (Stein et al., 1998; Preece et al., 2001; Luo et al., 2003; Kalisch et al., 2005). As such, these data support the continued use of the fMRI BOLD signal as tool for studying pharmacological effects. Furthermore, these data demonstrate the MDMA administration elicits a widespread activation of regions innervated by dopamine, supporting the role of this neurotransmitter in the neuropharmacology of MDMA.

It is somewhat surprising that cocaine did not elicit activation in the striatum. However, this is consistent with the effects of cocaine on neurometabolism (Henry-Kirkland et al., in press) and blood flow (Howell et al., 2009), in this species. The differential capacity of cocaine and MDMA to elicit striatal activation may be related to their differential mechanism of action. The pharmacological mechanism of action of cocaine includes reuptake inhibition whereas the mechanism of action of MDMA includes substrate based release of neurotransmitters (Sulzer et al., 2005). At present, it is unknown if the fMRI

BOLD signal is differentially sensitive to these mechanisms. Further research into this important question is warranted.

Alternatively, the differential capacity of MDMA and cocaine to elicit striatal activation, in these subjects, may be related to their drug history. At the time of these studies, all three subjects had a history of self-administering cocaine but not MDMA. Furthermore, this same drug history was present in the subjects of other studies to show a lack of effect of cocaine on striatal activity (Howell et al., 2009; Henry-Kirkland et al., in press). Previous experience self-administering drugs of abuse has a well documented modulatory influence on many of the acute effects of those drugs (Moore et al., 1998; Letchworth et al., 2001). Furthermore, these modulatory effects are often specific to the previously self-administered drug and do not cross generalize to other drugs of abuse. At present, it is unknown if the acute BOLD response to pharmacological challenge is also sensitive to the subjects drug history; however, the acute neurometabolic effects of cocaine measured via FDG PET imaging are (Henry-Kirkland et al., in press), suggesting that it is likely that the BOLD signal is also sensitive to this variable. Understanding how the acute response to drug challenge is modulated by drug exposure history is likely to advance our understanding of the changes that occur in the brain is response to drug exposure. As these changes likely play an important role in the long term effects of drug exposure, such as addiction, further research into this important topic is clearly warranted.

In addition to activation of dopaminergic regions, MDMA-elicited activation in regions that receive relatively serotonergic input but little, if any, dopaminergic

input. These regions included areas that may have relevance for the hallucinogenic effects of MDMA, such as primary auditory, somatosensory, and visual cortex. Furthermore, MDMA-elicited activation of regions, such as the amygdala and hypothalamus, that may have relevance for its “empathogenic” effects. This complex pattern of brain activation is clearly different from that produced by cocaine. Moreover, this dual activation of dopaminergic and serotonergic regions is consistent with previous findings of the neuroactivational effects of MDMA using fMRI in nonhuman primates (Brevard et al., 2006; Meyer et al., 2006). Furthermore, the finding of activation of both dopaminergic and serotonergic brain regions is consistent with neurometabolic effects of the closely related analog of MDMA methylenedioxyethylamphetamine (MDE) in humans (Gouzoulis-Mayfrank et al., 1999; Schreckenberger et al., 1999). Finally, it is consistent with direct measures the neurochemical effects of MDMA in nonhuman primates using *in vivo* microdialysis (Chapter 3). As such, these data demonstrate the MDMA administration elicits a widespread activation of regions innervated by serotonin, supporting the role of this neurotransmitter in the neuropharmacology of MDMA.

Previous studies suggest that the complexity of the neuropharmacological effects of MDMA may be mediated by qualitative differences in the effects of its stereoisomers. Consistent with its neurochemical effects (Chapter 3) and similarly to racemic MDMA, S(+)-MDMA-elicited activation of regions innervated by dopamine and serotonin. In contrast but consistent with its neurochemical effects (Chapter 3), R(-)-MDMA selectively, and dose-dependently, elicited

activation of regions that receive serotonin innervation but not dopamine innervation. It is important to note that regions that receive dopaminergic innervation also receive serotonergic innervation. Consistent with previous findings (A.J. Schwarz, 2004), this suggests that enhanced dopamine neurotransmission is required to activate regions that receive dopaminergic innervation. The concordance of these fMRI measurements with direct neurochemical measures across all three forms of MDMA further validate the use of BOLD fMRI as a tool for studying pharmacology. The neurophysiological underpinnings of the BOLD are unclear (Logothetis, 2003; Nair, 2005). Nevertheless, these data strongly suggest that the BOLD fMRI signal is modulated by the direct neurochemical effects of each drug. Furthermore, these results extend the direct neurochemical measurements of the stereoisomers of MDMA by demonstrating that the qualitative dissociation in their neuropharmacological effects is not limited to the striatum but occurs across the entire cerebrum. As such, these data strongly support the hypothesis that qualitative differences in its stereoisomers mediate the complex effects of racemic MDMA.

| Variable | Axis | No stimulation | Visual stimulation | Cocaine |
|---|---|---|---|---|
| **Maximum** | **X Translation** | 0.09 (0.02) | 0.17 (0.003) | 0.05 (0.03) |
| | **Y Translation** | 0.20 (0.07) | 0.24 (0.08) | 0.07 (0.06) |
| | **Z Translation** | 0.15 (0.06) | 0.33 (0.14) | 0.10 (0.09) |
| | **X Rotation** | 0.44 (0.17) | 0.91 (0.38) | 0.68 (0.08) |
| | **Y Rotation** | 0.39 (0.11) | 0.73 (0.11) | 0.70 (0.21) |
| | **Z Rotation** | 0.54 (0.06) | 0.54 (0.03) | 0.71 (0.21) |
| **Mean** | **X Translation** | 0.02 (0.005) | 0.03 (0.005) | 0.009 (0.007) |
| | **Y Translation** | 0.05 (0.01) | 0.06 (0.02) | 0.01 (0.01) |
| | **Z Translation** | 0.03 (0.01) | 0.07 (0.03) | 0.02 (0.01) |
| | **X Rotation** | 0.12 (0.03) | 0.18 (0.06) | 0.14 (0.02) |
| | **Y Rotation** | 0.07 (0.02) | 0.13 (0.03) | 0.08 (0.02) |
| | **Z Rotation** | 0.16 (0.03) | 0.17 (0.03) | 0.20 (0.05) |
| **Standard deviation** | **X Translation** | 0.02 (0.005) | 0.03 (0.004) | 0.008 (0.006) |
| | **Y Translation** | 0.04 (0.01) | 0.06 (0.02) | 0.01 (0.01) |
| | **Z Translation** | 0.03 (0.01) | 0.07 (0.03) | 0.02 (0.01) |
| | **X Rotation** | 0.09 (0.03) | 0.17 (0.06) | 0.12 (0.02) |
| | **Y Rotation** | 0.07 (0.02) | 0.13 (0.02) | 0.10 (0.03) |
| | **Z Rotation** | 0.12 (0.02) | 0.13 (0.02) | 0.16 (0.05) |

**Table 4-1.** Translational (mm) and rotational (degrees) motion parameters collected under three different scan conditions: the absence of stimulations, presentation of a visual stimulus, or administration of an acute intravenous bolus of cocaine (0.3 mg/kg).

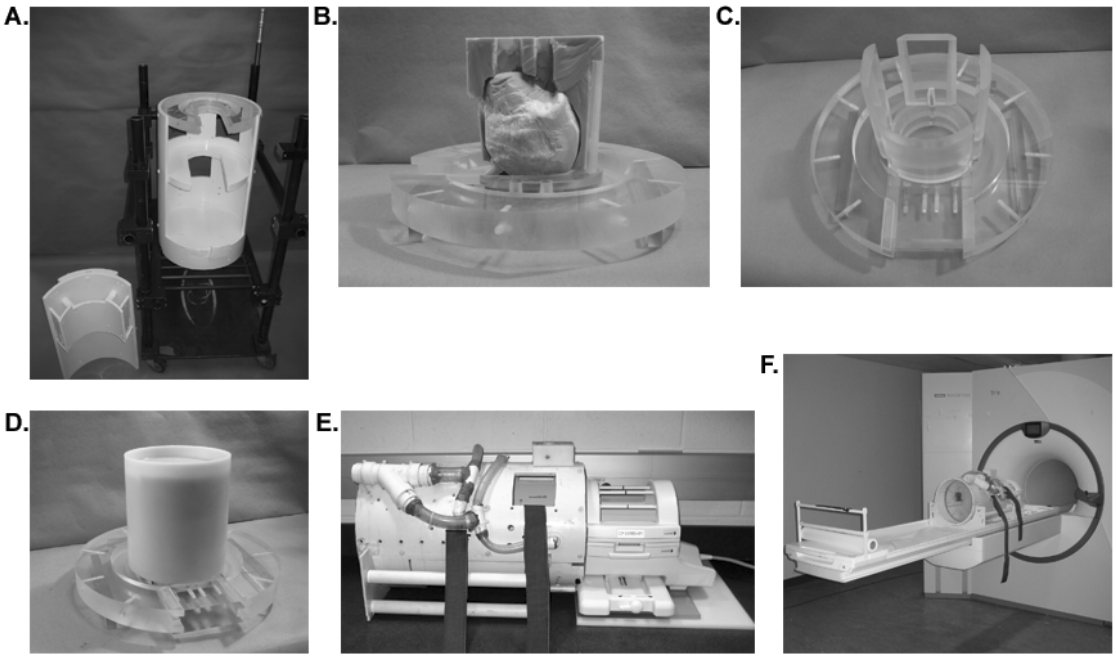


**Figure 4-1.** Pictorial representation of the apparatus developed to conduct fMRI studies in fully conscious rhesus monkeys. Panel A: the outer frame of the apparatus attached to a standard primate chair. Panel B: the top plate in side profile and a representative Plaster of Paris cast sitting on the top plate in the position that a subject would occupy in the apparatus (side profile of subject looking upwards). Panel C: three vertical acrylic plates that attach to the outside of the mold which augment its structural stability and allow for additional functionality. Panel D: the plastic tube that is placed over the head restraints to provide structural support and complete the head restraint process. Panel E: the entire apparatus shown in side profile in the position that it would occupy during a scan and attached to the Siemens transmit/receive volume coil used in this study Panel F: the entire apparatus on the bed of the Siemens 3 Trio scanner used in this study.

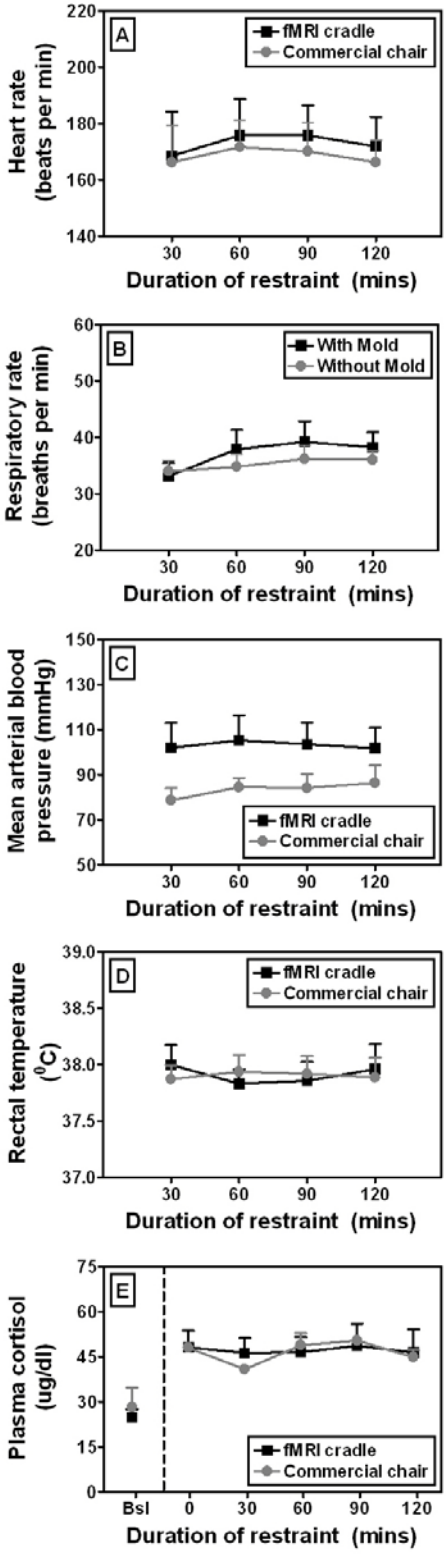


**Figure 4-2.** Effects of immobilization in the custom fMRI apparatus (closed squares) on rhesus monkey heart rate (A), respiratory rate (B), mean arterial blood pressure (C), rectal temperature (D), and plasma cortisol levels (E) in comparison to immobilization in a standard primate chair (closed circles) with the exception of respiratory rate which was compared to immobilization in the custom fMRI cradle in the absence of the custom fitted mold (see Methods). These metrics were stable across the session and did not significantly differ by condition. All points represent the group mean ± SEM. *Abscissae:* Time expressed in minutes in reference to the initiation of the session and plotted on a linear scale. *Ordinates:* Heart rate, respiratory rate, mean arterial blood pressure, rectal temperature, and plasma cortisol concentration expressed as absolute beats per minute, breaths per minutes, mmHg, degrees Celsius, or ug/dl, respectively.

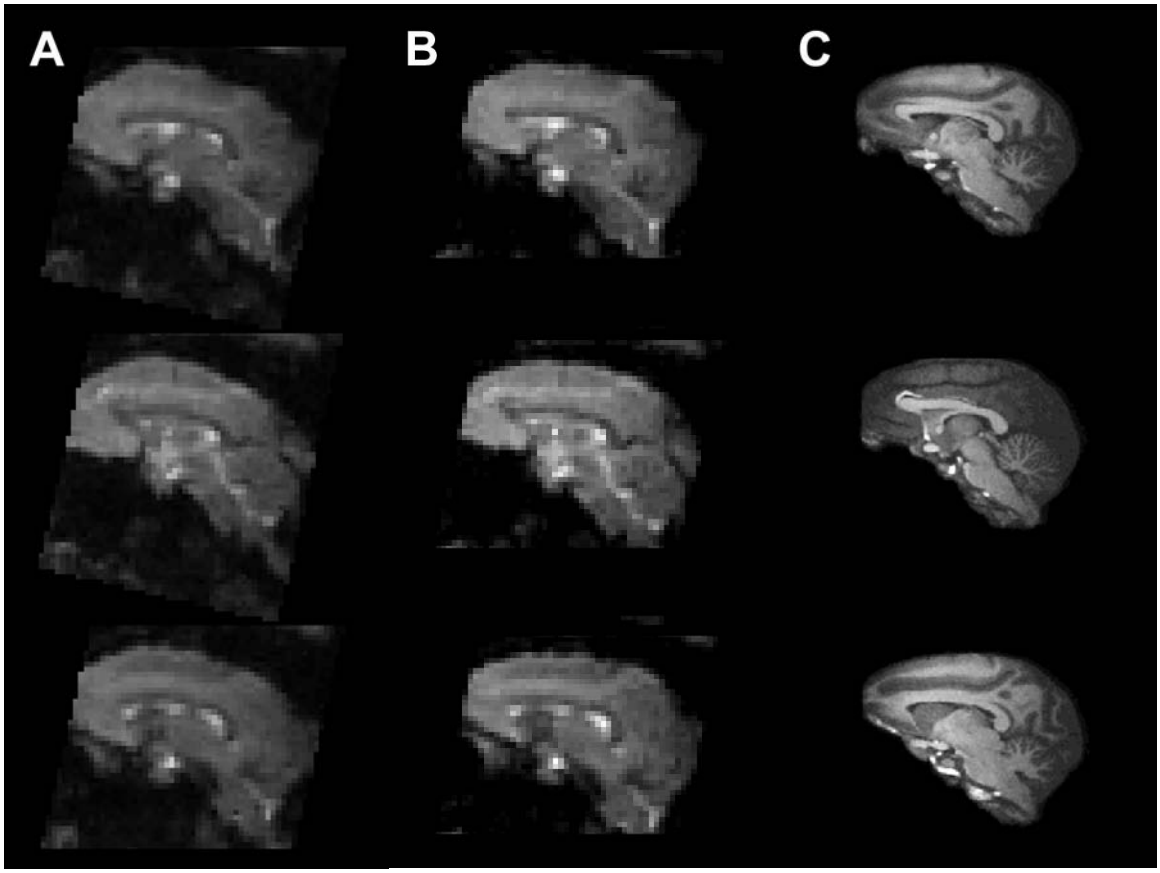


**Figure 4-3.** Effects of the described procedure to correct the geometric distortions that are inherent to EPI based imaging sequences using a collected map of the inhomogeneities in the main magnetic field ($B_0$). All images are presented in sagital sections. The central images were collected at the midline whereas the top and bottom images were collected 3 mm lateral to the midline. Panel A: raw uncorrected EPI images in this format. Panel B: the same images after correction for geometric distortions. Panel C: anatomical images collected from the same subject (while conscious) in the same format.

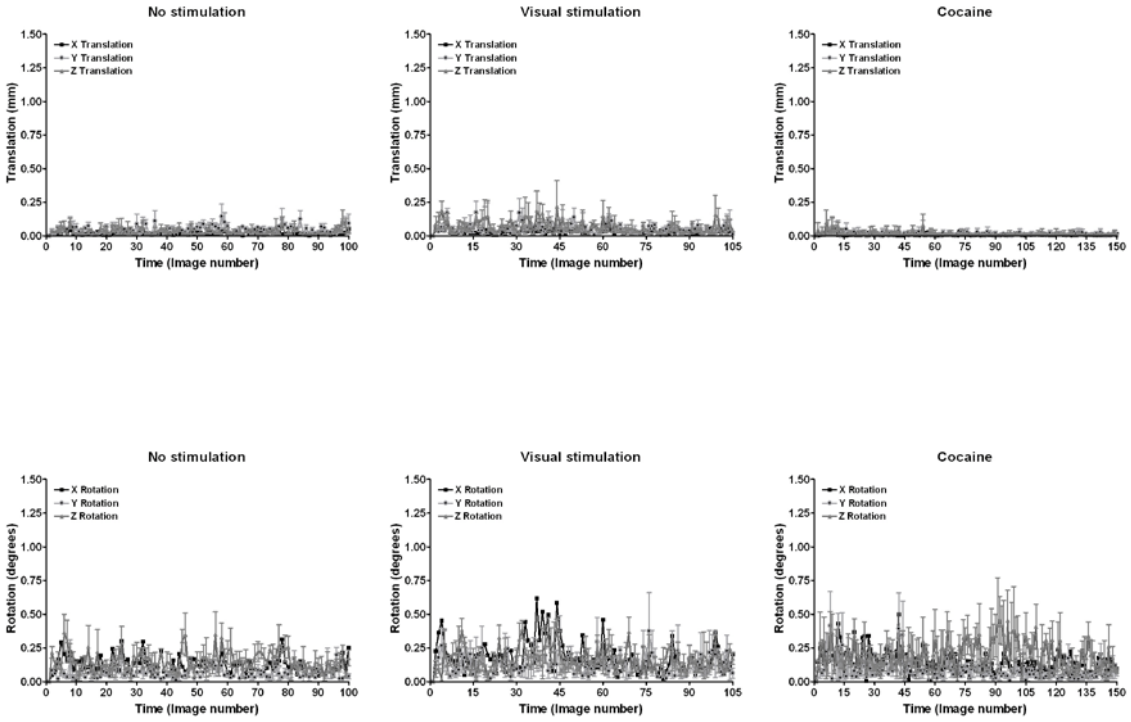


**Figure 4-4.** Absolute translational (top row) and rotational (bottom row) spatial movements measured along the X (closed squares), Y (closed circles), and Z (closed triangles) axes when the subjects were scanned without stimulation (left column), with visual presentation of a rotating checkerboard stimulus (center column), or with intravenous administration of 0.3 mg/kg cocaine (right column). The graphs are scaled to show a maximum of translation of one full voxel (1.5 mm) or a maximum rotation of 1.5 degrees as these represent commonly held standards for acceptable motion levels. All points represent the group mean ± SEM. *Abscissae:* Time expressed in acquisition number (TR) in reference to the initiation of the scan and plotted on a linear scale. *Ordinates:* Absolute translations (top row) or rotations (bottom row) from acquisition to acquisition expressed as millimeters or degrees respectively.

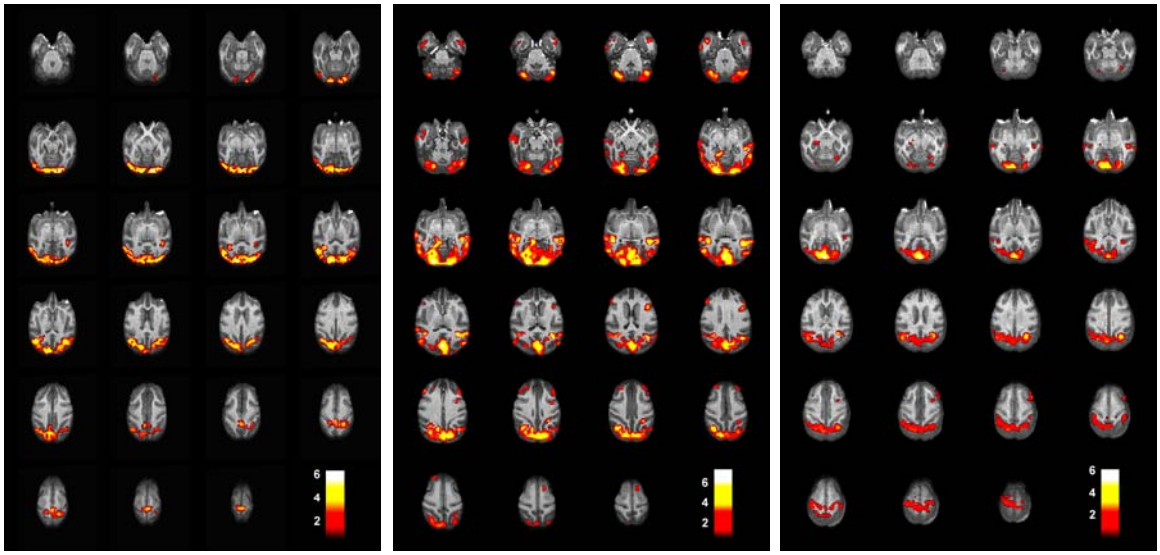


**Figure 4-5.** Effects of the visual presentation of a rotating checkerboard stimulus on blood oxygenation levels expressed as t-values of the significance of the change. All images are presented in transverse sections. Consistent with previous studies using a similar stimulus, the changes in blood oxygenation are principally localized to primary visual cortex. Each panel represents a separate subject. Only first-level analyses were carried out. The magnitude of the t-value is color coded as shown by the key inset in each panel.

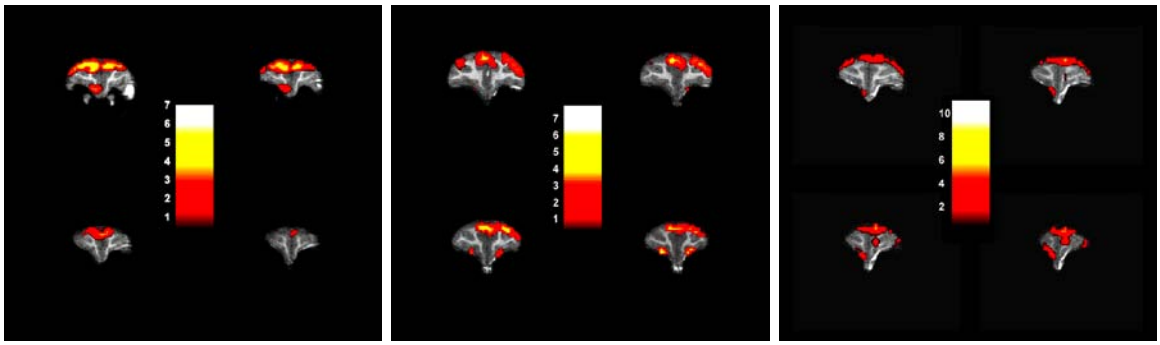


**Figure 4-6.** Effects of intravenous administration of cocaine (0.3 mg/kg) on blood oxygenation levels in the pre-frontal cortex expressed as t-values of the significance of the change. All images are presented in coronal sections. Consistent with previous studies, the changes in blood oxygenation are principally localized to anterior cingulate and dorsal regions of the pre-frontal cortex. Each panel represents a separate subject. Only first-level analyses were carried out. The magnitude of the t-value is color coded as shown by the key inset in each panel.

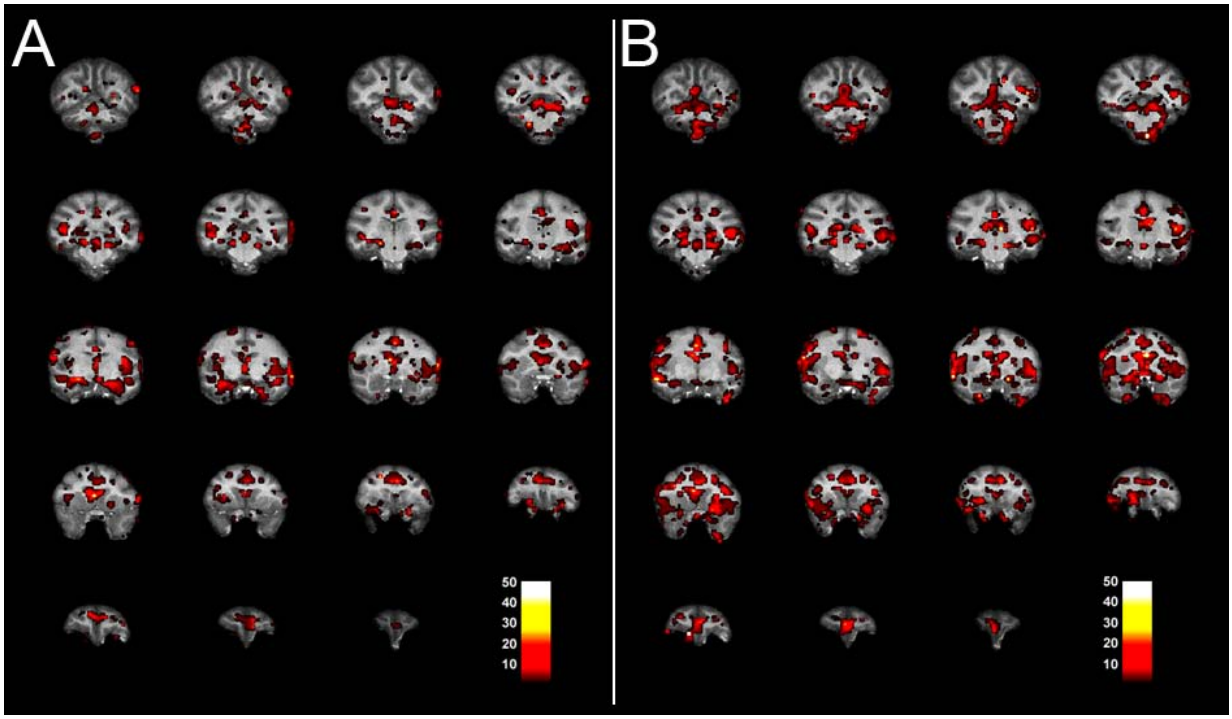


**Figure 4-7.** Effects of intravenous administration of S,R(+/-)-MDMA at 0.3 mg/kg (A) or 1.0 mg/kg (B) on blood oxygenation levels expressed as t-values. Images are presented as coronal sections in radiological convention. Each panel represents group data. The magnitude of the t-value is color coded as shown by the key inset in each panel.

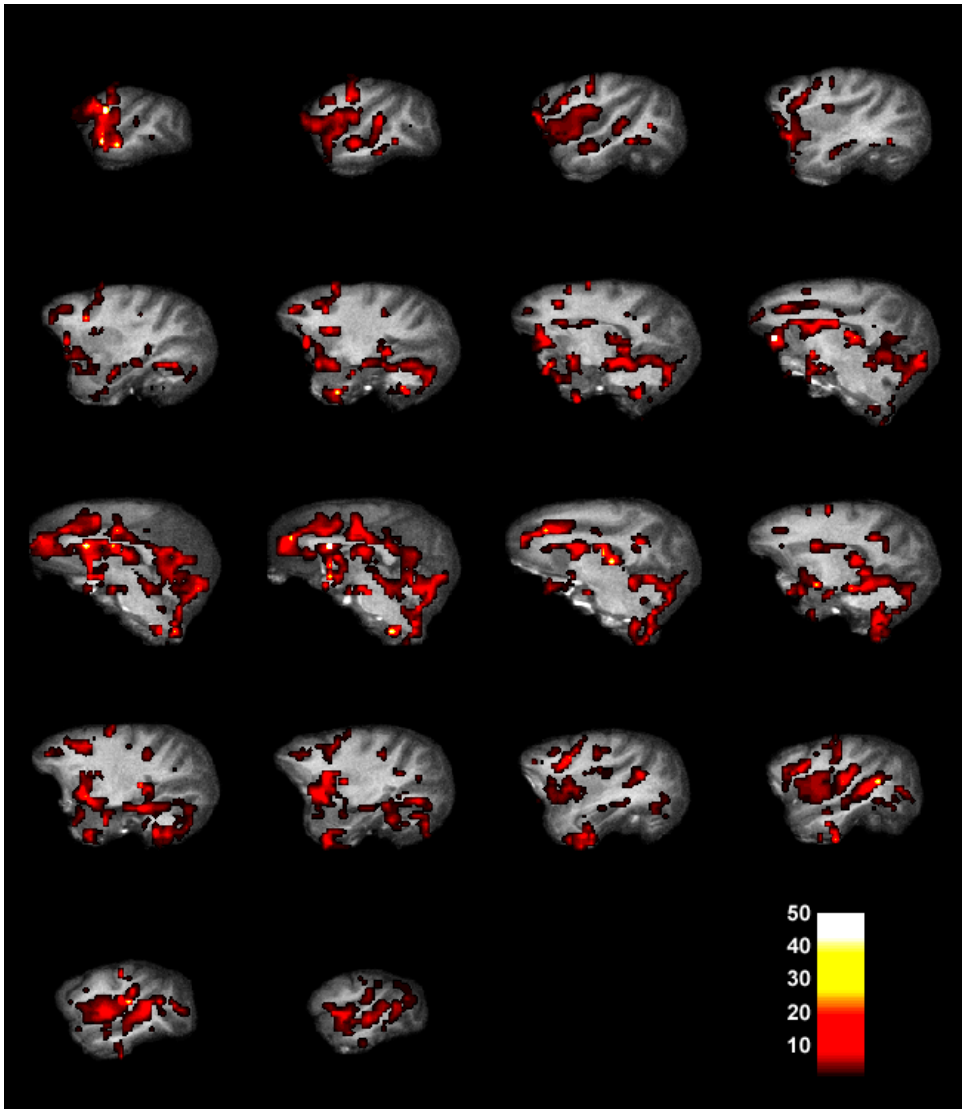


**Figure 4-8.** Effects of intravenous administration of S,R(+/-)-MDMA at 1.0 mg/kg on blood oxygenation levels expressed as t-values. Data are replotted from figure 4-7 but presented as sagital sections to illustrate the mixture of dopaminergic and serotonergic effects engendered by S,R(+/-)-MDMA. The magnitude of the t-value is color coded as shown by the key inset in each panel.

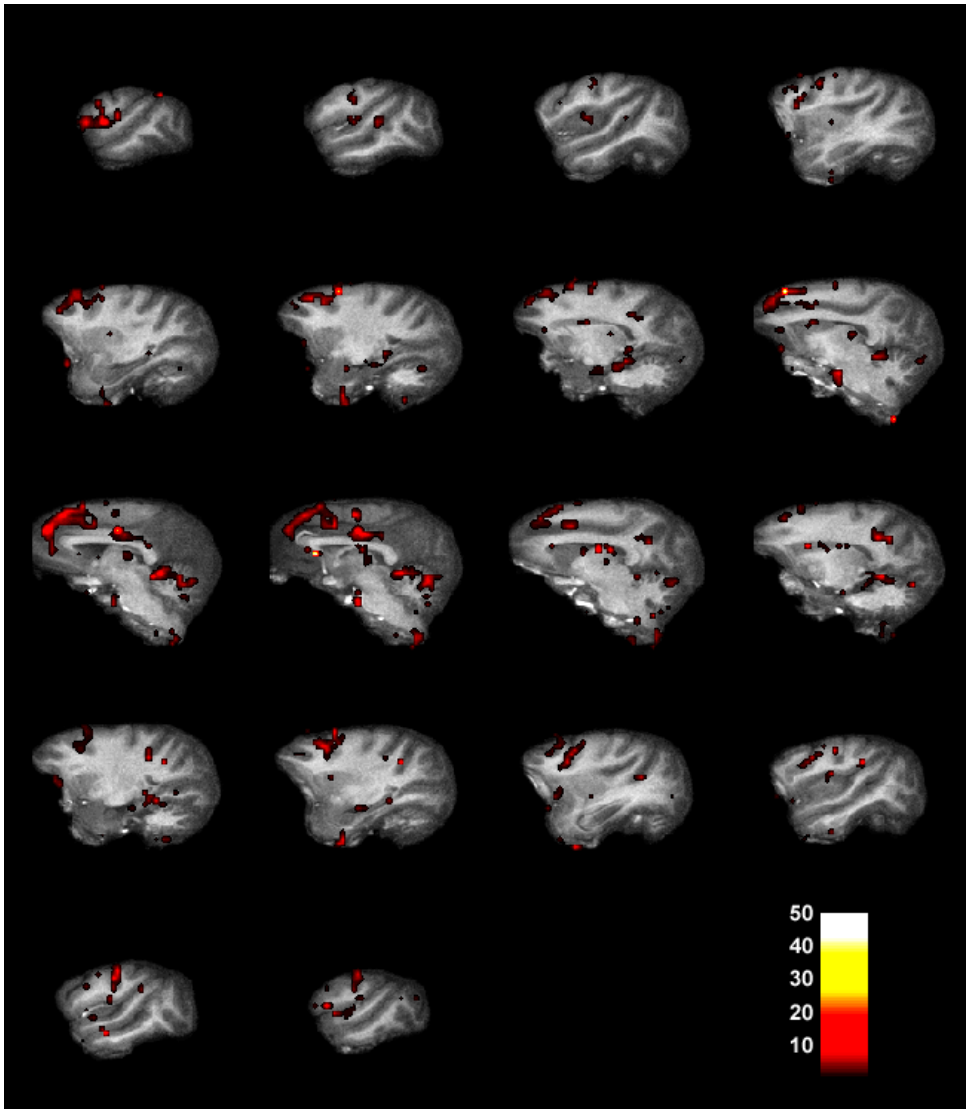


**Figure 4-9.** Effects of intravenous administration of cocaine at 0.3 mg/kg on blood oxygenation levels expressed as t-values. Data are replotted from figure 4-7 but presented as sagital sections to illustrate the selective dopaminergic effects engendered by cocaine. Data represent the entire group. The magnitude of the t-value is color coded as shown by the key inset in each panel.

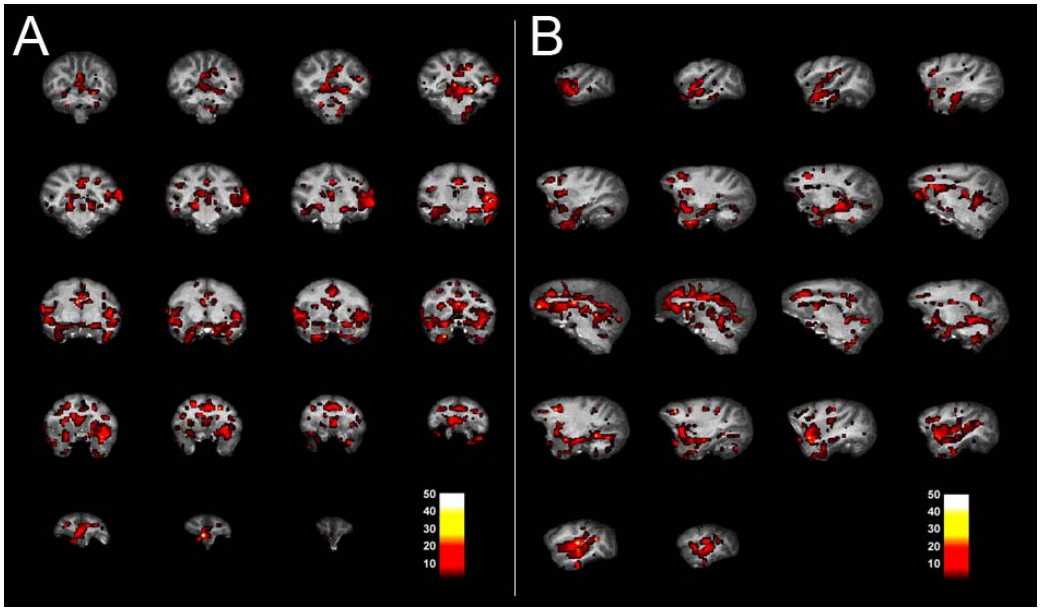


**Figure 4-10.** Effects of intravenous administration of S(+)-MDMA at 0.3 mg/kg on blood oxygenation levels expressed as t-values. Images are presented as both coronal (A) and sagital (B) sections in radiological convention. Each panel represents group data. The magnitude of the t-value is color coded as shown by the key inset in each panel.

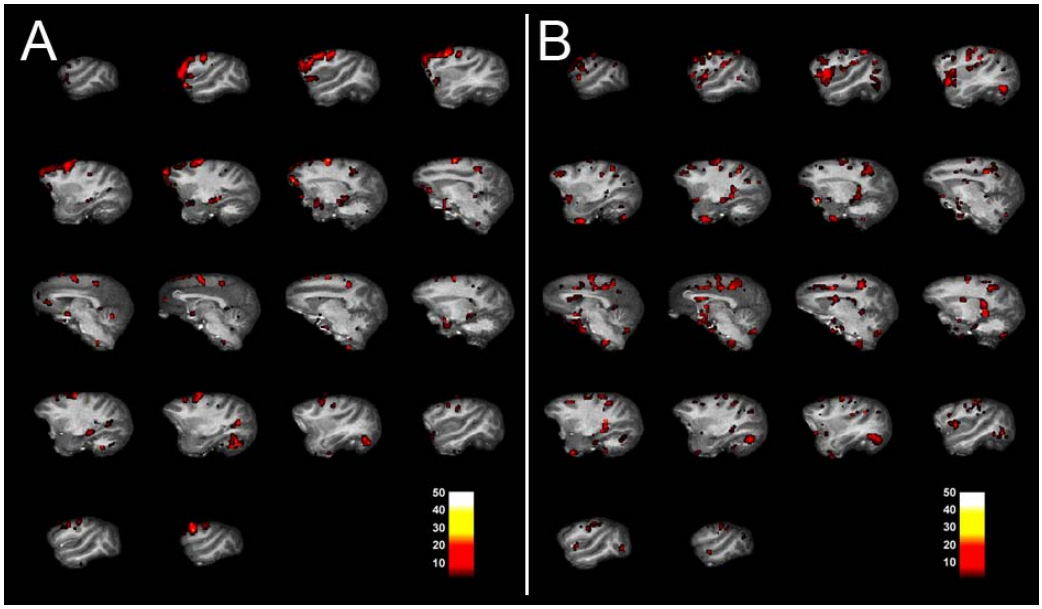


**Figure 4-11.** Effects of intravenous administration of R(-)-MDMA at 0.3 mg/kg (A) or 1.0 mg/kg (B) on blood oxygenation levels expressed as t-values. Images are presented as sagital sections in radiological convention. Each panel represents group data. The magnitude of the t-value is color coded as shown by the key inset in each panel.

# Chapter 5

## General discussion

### Summary of findings

MDMA abuse is widespread and has been associated with acute toxic effects including lethality and sustained effects indicative of brain damage. MDMA defies categorization into traditional classification schemes for psychoactive drugs as its biological effects appear to be a complex mixture of psychomotor-stimulant-like and hallucinogen-like effects. The “stereoisomeric hypothesis” of MDMA’s complex effects proposes that these complex effects are mediated by qualitative differences in the effects of its stereoisomers. Specifically, S(+)-MDMA is proposed to more readily function as a psychomotor-stimulant whereas R(-)-MDMA is proposed to more readily function as a hallucinogen. Support for this hypothesis comes from several levels of analysis, including differential binding to monoamine transporters and receptors, differential effects on dopamine release, and differential interoceptive and behavioral effects (Anderson et al., 1978; Glennon et al., 1988; Battaglia and De Souza 1989; Baker et al., 1995; Lyon et al., 1986; Nash et al., 1994; Setola et al., 2003; Acquas et al., 2007; Nichols, et al., 1982; Fantegrossi et al., 2003; Fantergrossi et al., 2005) particularly in rodent models. However, this hypothesis is controversial as all known chiral drugs possess stereoisomers that exhibit a potency but not an apparent efficacy difference. In other words, each stereoisomer produces the same effects but the concentration required to produce those effects differs. To date, no pharmacotherapeutic has been shown

to be effective in the treatment of MDMA abuse. Thoroughly understanding the mechanisms that mediate its complex biological effects may aid the development of novel treatment strategies for MDMA abuse and its long-term deleterious consequences. To this end, in the present experiments, we have sought to further explore the *in vivo* neuropharmacology of MDMA and its stereoisomers. The key findings of this dissertation project are summarized below:

1) Across species and assay, the stereoisomers of MDMA have qualitatively different interoceptive and behavioral effects. The interoceptive effects of the stereoisomers of MDMA are distinct yet overlap. The interoceptive effects of each stereoisomer appears to have a serotonergic component whereas only the S(+) stereoisomer has a dopaminergic component to its interoceptive effects. The dopaminergic component appears to be most similar to drugs that - through substrate based release - function as indirect agonists such as the psychomotor-stimulant amphetamine. In contrast, R(-)-MDMA appears to have interoceptive effects, lacking in S(+)-MDMA, that are similar to hallucinogenic agonists of the 5-$HT_{2A}$ receptor. Consistent with these effects, racemic and S(+)-MDMA disrupt sleep in rhesus monkeys whereas R(-)-MDMA does not. S,R(+/-)-MDMA increased the latency of the subjects to fall asleep but did not significantly decrease the duration of sleep. S(+)-MDMA increased sleep latency and decreased sleep duration. R(-)-MDMA did not significantly alter any sleep parameter measured.

2) MDMA elicits a complex mixture of endocrine and neurochemical effects which segregate coherently across its steroisomers. S,R(+/-)-MDMA elicits release of dopamine and serotonin and secretion of prolactin in rhesus monkeys. S(+)-MDMA elicits release of dopamine and serotonin but does not elicit secretion of prolactin. R(-)-MDMA elicits secretion of prolaction and release of serotonin but not release of dopamine.

3) BOLD fMRI is a valid assay for measuring whole brain *in vivo* neuropharmacological effects. The BOLD fMRI signal appears to be sensitive to the basic parameters that control drug action. The BOLD fMRI response is determined by the dose administered and the specific neurochemical effects of the drug administered. In other words, it is controlled by the pharmacodynamic parameters of drug action.

4) MDMA elicits a complex pattern of neuroactivational effects which segregate coherently across its steroisomers. S,R(+/-)-MDMA elicits increased blood oxygenation in brain regions innervated by both dopamine and serotonin projection neurons. S(+)-MDMA elicits a similar pattern of activation. In contrast, the effects of R(-)-MDMA appears to elicit activation of regions innervated by serotonin neurons but not dopamine neurons.

5) The 5-$HT_{2A}$ receptor and the serotonin transporter are viable targets for treating MDMA abuse. Antagonism of the 5-$HT_{2A}$ receptor attenuated the

behavioral and dopaminergic effects of MDMA. Administration of a SSRI attenuated prolactin secretion elicited by S,R(+/-)-MDMA and serotonin release by R(-)-MDMA. However, combined treatment with a 5-$HT_{2A}$ receptor antagonist was required to attenuate prolactin secretion elicited by R(-)-MDMA.

Relevance for the study of MDMA

The present studies provide substantial support for the "stereoisomeric hypothesis" of MDMA's complex effects. At the outset of these studies, significant evidence in support of this hypothesis has come from studies utilizing drug-discrimination procedures. The results of these studies generally showed marked differences in the interoceptive effects of each stereoisomer of MDMA. For example, S(+)-MDMA fully substituted for the interoceptive cue produced by the phenethylamine stimulant amphetamine but did not substitute for the phenethylamine hallucinogen DOM (Glennon et al., 1988). However, the results of other studies were in apparent contrast to these findings as, for example, cocaine substituted for the interoceptive effects of either S(+)-MDMA or R(-)-MDMA (Bondareva et al., 2005). The collective results across these studies suggested to us that the discrepancies between studies may be related to the nature of the comparison drugs. Specifically, these results suggested that a dissociation in the interoceptive effects of each stereoisomer of MDMA was more likely to be found if the comparison drugs used were chemically and pharmacologically similar to MDMA. The results of the present studies support this supposition. The ability of these subjects to discriminate the interoceptive

effects of amphetamine from cocaine or DPT from 2C-T-7 indicates that these drug-discrimination procedures have the capacity to train subjects to discriminate quite subtle differences in pharmacological effects. This supports previous findings that drug-discrimination procedures are highly selective and sensitive (Schuster and Johanson, 1988; Brauer et al., 1997). Furthermore, it indicates that care should be taken in establishing any single positive control drug as an archetype for drug-discrimination procedures. In other words, care should be taken in the design of these procedures as results with a drug considered representative of its class, such as cocaine, may not generalize to other members of this class. This may have particular relevance for a drug with complex effects such as MDMA.

While the results of these experiments may explain the discrepancies across MDMA drug-discrimination studies, it is important to recognize that they also generally support the results of *individual* studies. For example, consistent with the results reported by Bondareva and colleagues (2005), cocaine substituted for both S(+)-MDMA and R(-)-MDMA. However, a study utilizing a parametric design that varied the chemical and pharmacological similarity of the test compounds to MDMA, in the context of drug-discrimination, had never been carried out before. The results of the present study indicate that each stereoisomer has a serotonergic component to its interoceptive effects. Furthermore, S(+)-MDMA has a dopaminergic component to its interoceptive effects that is lacking in its R(-) counterpart. These results may have explanatory power for the previous finding that each stereoisomer substitutes for the other

(Fantegrossi et al., 2009) yet appears to have distinct interoceptive effects (Chapter 2). In addition, these data should largely bring closure to significant debate in regards to whether the stereoisomers of MDMA engender distinct interoceptive effects in preclinical models as this has now been demonstrated across different operant schedules, training procedures, training doses, training drugs (generalization versus substitution), and species. However, a single antiquated and loosely designed study (Anderson et al., 1978) is the only published description of the subjective effects of the stereoisomers of MDMA in humans. Future studies that determine these subjective effects using established methods would further our understanding of the complex effects of MDMA.

The present experiments were also designed to shed new light on the behavioral effects of MDMA in nonhuman primates. Previous studies had shown that MDMA reliably elicits locomotor-stimulant effects in rodents (Slikker et al., 1989; Spanos and Yamamoto, 1989; Callaway et al., 1990; McNamara et al., 1995; De Souza et al., 1997; Fantegrossi et al., 2003; Fantegrossi et al., 2004a; Acquas et al., 2007) but did not exhibit locomotor-stimulant effects in rhesus monkeys (Taffe et al., 2006) or behavioral-stimulant effects in squirrel monkeys (Fantegrossi et al., 2009). This raised the possibility that MDMA does not engender stimulant-like effects in nonhuman primates. Sleep disruption is a hallmark effect of psychomotor-stimulants that we predicted would be likely to be elicited by MDMA. We made this prediction based on the effects of MDMA in humans (Randall et al., 2009). These studies demonstrate that, as predicted, MDMA disrupts sleep in nonhuman primates. In combination with studies

showing that MDMA is self-administered by nonhuman primates, these results should largely resolve any question as to whether or not MDMA elicits stimulant-like effects in this taxa. It is important to note, however, that the sleep disruption elicited by MDMA was modest in comparison to amphetamine. Future studies should determine the underlying mechanisms for this difference as it may inform our understanding of sleep regulation.

Further experiments were carried out to examine the "stereoisomeric hypothesis" of MDMA's complex effects in nonhuman primates. In rodents, S(+)-MDMA elicits locomotor-stimulant effects whereas R(-)-MDMA does not (Fantegrossi et al., 2003; Acquas et al., 2007). Therefore, we predicted that S(+)-MDMA but not R(-)-MDMA would disrupt sleep in rhesus monkeys. The results of the present experiments confirmed this prediction. Collectively, this work demonstrates that S(+)-MDMA more readily functions as a psychomotor-stimulant than R(-)-MDMA in different species and under diverse conditions. However, to date, no studies have determined if the stereoisomers of MDMA differentially elicit hallucinogen-like effects in nonhuman primates. To this end, an examination of the effects of each form of MDMA on pre-pulse inhibition of startle may be informative. Nevertheless, there is now considerable evidence that the stereoisomers of MDMA engender qualitatively different behavioral and interoceptive effects.

Once these qualitatively different behavioral and interoceptive effects were established, we proceeded to characterize the underlying neuropharmacological mechanisms for these differential effects. Experiments were undertaken to

determine the neuropharmacological effects of each form of MDMA using *in vivo* microdialysis, plasma prolactin analysis, and fMRI. We believed these procedures would strongly complement one another as they allowed for a direct measurement at a site of action, a systemic measurement, and a whole brain measurement. *In vivo* microdialysis revealed that only S(+)-MDMA and S,R(+/-)-MDMA-elicited dopamine release. Furthermore, S(+)-MDMA had more pronounced effects on dopamine release than the racemate which is consistent with findings from rodents using similar procedures (Hatzidimitriou et al., 2002; Acquas et al., 2007). In contrast, all three forms of MDMA elicited serotonin release. This pattern is one that would be predicted to produce the behavioral and interoceptive effects previously described. As such, these data indicate that the qualitatively different behavioral and interoceptive effects elicited by the stereoisomers of MDMA are mediated by qualitatively different neuropharmacological effects.

These experiments were then extended by determining the effects of each form of MDMA on prolactin secretion. These procedures were designed to measure the integrated effect of each form of MDMA across the entire system rather than the site-directed approach taken with microdialysis. Initial experiments, using a combination of selective positive control drugs and concurrent sampling procedures, verified that prolactin measures are a valid systemic index of the relative tone of dopaminergic versus sertonergic neurotransmission. Furthermore, additional experiments demonstrated that blockade of the serotonin transporter attenuated S,R(+/-)-MDMA-elicited prolactin

secretion. Previous studies suggested that the relative tone of dopaminergic versus sertonergic neurotransmission controls prolactin release ((Aloi et al., 1984; Baumann et al., 2008) but these data are the first direct evidence of this relationship. These studies also showed that R(-)-MDMA and S,R(+/-)-MDMA elicit prolactin secretion whereas S(+)-MDMA does not. Importantly, these results support the findings of the microdialysis experiments in that they were consistent with R(-)-MDMA functioning as a selective serotonin releaser whereas S(+)-MDMA has mixed effects on serotonin and dopamine release.

While the results of the prolactin experiments provide substantive support for the findings of the microdialysis experiments, they also raised some important questions. First, pretreatment with an SSRI attenuated prolactin secretion by S,R(+/-)-MDMA. This is consistent with previous findings that SSRIs attenuate S,R(+/-)-MDMA serotonin release (Gudelsky and Nash 1996; Mechan et al., 2002). Indeed, the interpretation of previous findings that SSRIs attenuate S,R(+/-)-MDMA-elicited behavioral effects have been widely interpreted to indicate that those effects are directly mediated by serotonin. If SSRIs also attenuate MDMA-elicited prolactin secretion and prolactin may mediate some of the behavioral effects of MDMA this interpretation may not be tenable. In support of this contention, recent reports indicate that the so called “empathogenic” effects of MDMA may be mediated by eliciting secretion of the hormone oxytocin (Dumont et al., 2000). Dissociating the relative contribution of serotonin and prolactin to the behavioral and interoceptive effects of MDMA may not be straightforward but could be accomplished through the use of selective receptor

antagonists. Studies such as this may further enhance our understanding of the neuropharmacology of MDMA and perhaps other psychoactive drugs.

Second, attenuation of R(-)-MDMA-elicited prolactin secretion required cotreatment with an SSRI and a 5-$HT_{2A}$ receptor antagonist. These data suggest, consistent with previous findings (Nash et al., 1993; Setola et al., 2003), that *in vivo* R(-)-MDMA can function as both a serotonin releaser and a 5-$HT_{2A}$ receptor agonist. While important for understanding the neuropharmacology of R(-)-MDMA, it is perplexing that this did not appear to be also true for S,R(+/-)-MDMA as S,R(+/-)-MDMA is partially composed of R(-)-MDMA. A straightforward explanation may be a potency difference at the 5-$HT_{2A}$ receptor. S,R(+/-)-MDMA overcame the effects of the SSRI at higher doses. We interpreted this effect as likely being mediated through competition at the transporter but it is possible that it was mediated through S,R(+/-)-MDMA reaching a sufficient concentration to function as an agonist of the 5-$HT_{2A}$ receptor at this dose and thereby circumvent the effects of the SSRI. On the other hand, if this effect is not mediated through a simple potency difference it could be due to some *in vivo* interaction of S(+)-MDMA with R(-)-MDMA preventing R(-)-MDMA from agonizing the 5-$HT_{2A}$ receptor when they are coadministered in the racemic mixture. Interestingly, this could not be through a direct interaction as we have found that S(+)-MDMA has no measurable affinity for the 5-$HT_{2A}$ receptor. To this author's knowledge, this would be the first example of a stereoisomer modifying its counterpart's effects at a receptor through an indirect mechanism. Further research is warranted.

The microdialysis and prolactin experiments were extended by determining the whole brain neuropharmacological effects of each form of MDMA using BOLD fMRI. In these studies, S(+)-MDMA and S,R(+/-)-MDMA-elicited activation in brain regions that receive either dopaminergic or serotonergic innervation. In contrast, the neuroactivational effects of R(-)-MDMA were confined to regions that receive serotonergic innervation but not dopaminergic innevation. These effects are consistent with the neurochemical and prolactin studies and previous neuroimaging studies of S,R(+/-)-MDMA or related compounds (Gouzoulis-Mayfrank et al., 1999; Schreckenberger et al., 1999; Brevard et al., 2006). Collectively, this provides compelling evidence for the "stereoisomeric hypothesis" of the complex effects of MDMA. Furthermore, these results highlight the strength of a multipronged approach to studying neuropharmacology. Finally, these distinct brain activation patterns are consistent with neural circuitry that one could expect to underlie the kinds of behavioral effects we measured.

In addition to further elucidating the neurophamacology of MDMA, these fMRI studies serve to further validate the use of BOLD fMRI as an assay for studying neuropharmacology. Other fMRI modalities have been somewhat validated for use in this field (Reese et al., 2000; Marota et al, 2000). However, despite achieving the highest implementation rates of all fMRI modalities, there are a paucity of studies validating BOLD fMRI in neuropharmacology (Stein et al, 1998; Kalisch et al, 2004; Luo et al, 2003; Preece et al, 2001). In the present experiments, the BOLD fMRI signal was both dose-dependent and controlled by

the selective pharmacological effects of each form of MDMA. This suggests that basic principles of pharmacological action control the BOLD signal response to drug administration. Indeed, especially now that it has been further validated, BOLD fMRI could be a powerful tool for determining the neurobiology of the complex subjective effects elicited by MDMA. For example, studies, similar to those that have been carried out with cocaine (Breiter et al., 1997, Kufahl et al., 2005), could determine the relationship between the subregional temporal dynamics of the BOLD fMRI signal and MDMA-elicited subjective effects. These studies could be particularly informative if combined with the use of selective antagonists. Attenuation of specific MDMA-elicited subjective effects and concomitant attenuation of a specific component of the BOLD fMRI response to MDMA by a selective antagonist would provide strong evidence for understanding the basic neurobiology of those subjective effects. As an extension, studies such as these could further our understanding of the neurobiology of mood and mood disorders in general.

While the present experiments enlighten our understanding of the neuropharmacology of MDMA and its stereoisomers, some behavioral effects of the stereoisomers are resistant to interpretation using this data set. For example, the dose-effect curves of S(+)-MDMA and S,R(+/-)-MDMA to increase sleep latency are basically identical. The effects of R(-)-MDMA did not reach statistical significance on this measure and visual inspection of the data suggests that this stereoisomer is, at best, considerably less potent at increasing sleep latency than S(+) and S,R(+/-)-MDMA. This indicates that there is an *in vivo* interaction

between the stereoisomers that enhances that effects of S,R(+/-)-MDMA beyond what would be expected from their isolated effects. A similar interaction has been described for the locomotor-stimulant effects of S,R(+/-)-MDMA in mice (Fantegrossi et al., 2003). As such, a full understanding of the effects of MDMA must take into account these interactions. Based on the pattern of effects across all three forms of MDMA and the concomitant attenuation of sleep disruption and dopamine release by selective antagonism of the 5-$HT_{2A}$ receptor, it seems reasonable to speculate that the sleep-disrupting effects of MDMA are mediated by dopamine neurotransmission. Furthermore, other work has shown that the locomotor-stimulant effects of psychomotor-stimulants, including MDMA, are also mediated by enhanced dopamine neurotransmission (Cabib et al., 1991; Bubar et al., 2004). However, in the present experiments we found that coadministration of R(-)-MDMA did not modulate dopamine release by S(+)-MDMA. As such, these data indicate that an interaction that alters the presynaptic elements of dopamine neurotransmission does not account for the enhanced behavioral effects of racemic MDMA. Therefore, we suggest that these enhanced behavioral effects may be mediated by a postsynaptic interaction of the stereoisomers of MDMA, presumably at postsynaptic dopamine receptors.

Given our conceptual model that the stereoisomers of MDMA are interacting at postsynaptic dopamine receptors, we have recently determined the binding affinities of each form of MDMA at many central receptors. Interestingly, we found that S(+)-MDMA exhibits appreciable affinity for dopamine D1 receptors (Table 5-1) when tested alone, however, coadministration of R(-)-MDMA

completely eliminated the affinity of S(+)-MDMA for D1 receptors. Of particular interest, R(-)-MDMA exhibited no affinity for D1 receptors in its own right, suggesting that R(-)-MDMA inhibition of S(+)-MDMA D1 receptor binding is mediated through an indirect and perhaps allosteric mechanism. Whether S(+)-MDMA is a full agonist, partial agonist, neurtral antagonist, or inverse agonist at the D1 receptor is not currently known. However, a partial agonist – relative to dopamine – or antagonist effect of S(+)-MDMA that is eliminated by coadministration of R(-)-MDMA could account for the described *in vivo* behavioral interactions.

In this conceptual model, S(+)-MDMA elicits locomotor-stimulant effects and sleep disruption through dopamine release. However, it partially attenuates the effectiveness of its own dopamine releasing effects through antagonist or partial agonist effects at postsynaptic D1 receptors. When coadministered with R(-)-MDMA S(+)-MDMA loses its affinity for D1 receptors and therefore the behavioral effects of racemic MDMA are enhanced. This could be described as a disinhibiting effect of R(-)-MDMA. While speculative, this model is supported by data showing that the locomotor-stimulant effects of MDMA are mediated by D1 receptors (Bubar et al., 2004). Indeed, it may also account for the results of the drug interaction studies. In both the present report and previous results (Fantegrossi et al., 2002), the behavioral effects of S(+)-MDMA were less sensitive than those of racemic MDMA to disruption by 5-$HT_{2A}$ receptor antagonism. If, unlike racemic MDMA, S(+)-MDMA can function as a partial agonist of D1 receptors then it is not surprising that its behavioral effects are less

sensitive to 5-$HT_{2A}$ receptor antagonism, as the present data set indicates that antagonism of this receptor attenuates MDMA-elicited behavioral effects by attenuating MDMA-elicited release of dopamine. Therefore, while speculative, this model accounts for many of the heretofore unexplained effects of MDMA.

While this conceptual model may account for many of the previously unexplained effects of MDMA, it must be further refined to account for some important findings with R(-)-MDMA. There are two published studies that examined self-administration of the stereoisomers of MDMA. Both utilized rhesus monkeys as subjects. However, in one study all three forms of MDMA maintained self-administration (Fantegrossi et al., 2002) whereas in the other only S(+) and S,R(+/-)-MDMA maintained self-administration (Wang and Woolverton 2007). There is little in the present data set that would account for self-administration of R(-)-MDMA under any conditions, as selective serotonin releasers and direct agonists of the 5-$HT_{2A}$ receptor are purportedly not self-administered by laboratory animals (Poling and Bryceland, 1979). However, our postsynaptic interaction conceptual model may also account for R(-)-MDMA self-administration. Since coadministration of R(-)-MDMA eliminates the affinity S(+)-MDMA for D1 receptors, it is reasonable to speculate that R(-)-MDMA can alter the conformational state of D1 receptors. While this would reduce the affinity of S(+)-MDMA for D1 receptors it could concurrently increase the affinity of dopamine for these same receptors. Indeed, it has been reported that dopamine receptors can exist in different conformational states and that dopamine has differential affinity for these states (for a review see Seeman et al., 2006). The

one region that receives extensive dopaminergic input found to be activated by administration of R(-)-MDMA in the fMRI experiments was the striatum, a region with a well established role in drug self-administration (Haber et al., 2010). Even at higher doses, R(-)-MDMA had no overt effects on dopamine release and, as such, dopamine release cannot account for striatal activation by R(-)-MDMA. As such, if R(-)-MDMA could trigger a mesocortical signal via altering the sensitivity of D1 receptors for their endogenous ligand dopamine, this could yield both striatal activity and support self-administration. It is important to note that this element of the model is also testable, as a determination of the affinity of dopamine for D1 receptors in the presence or absence of R(-)-MDMA should reveal whether or not R(-)-MDMA has the capacity to alter the affinity state of this receptor. In addition to finding that S(+)-MDMA has appreciable and relevant affinity for D1 receptors and that coadministration of R(-)-MDMA eliminates the affinity of S(+)-MDMA for these receptors, we have recently found that MDMA and its stereoisomers have distinct and relevant affinity for the 5-$HT_7$ and noradrenergic $\alpha_{2B}$ (Table 5-1). While there is a paucity of studies, some evidence suggests that these receptors may have a role in the behavioral and neurochemical effects of psychomotor-stimulants (Munzar and Goldberg, 1999). As such, we suggest that future studies should be designed to determine the *in vivo* effects of MDMA and its stereoisomers at central receptors, as this may lead to a unified understanding of the complex effects of MDMA.

While the present experiments may not explain the entirety of MDMA's effects, they represent a significant expansion of our understanding of the

neuropharmacology of MDMA. In particular, these studies collectively provide compelling evidence for the “stereoisomeric hypothesis” of the complex effects of MDMA. In this work, this hypothesis was not falsified at multiple levels of analysis. In combination with previous reports, we can now be confident that the stereoisomers of MDMA exhibit qualitatively different molecular, cellular, systems, and behavioral effects and that many of these effects hold true under widely divergent conditions. As such, it is reasonable to conclude that qualitative differences in its stereoisomers mediate the complex biological effects of racemic MDMA.

Abuse of MDMA is high risk behavior that has been associated with acute untoward effects including death. Currently, no pharmacotherapeutics are available for the treatment of MDMA abuse. It is possible that a drug that attenuates the behavioral and neuropharmacological effects of MDMA in preclinical models will be of utility in the treatment of MDMA abuse. To this end, in the present experiments, we evaluated the role of the 5-$HT_{2A}$ receptor in sleep disruption and dopamine release by MDMA. In these experiments, antagonism of this receptor attenuated both effects of MDMA. This is consistent with the effects of this receptor in rodent models (Bubar and Cunningham 2006). Furthermore, in combination with previous reports, antagonism of this receptor has now been shown to attenuate MDMA self-administration and MDMA-elicited sleep disruption and dopamine release. Importantly, antagonism of this receptor also attenuated sleep disruption and dopamine release by amphetamine. This is exciting as it suggests that the 5-$HT_{2A}$ receptor may be a viable target for

attenuating abuse of other stimulant-type drugs. Further support for this possibility has been garnered by studies demonstrating that antagonism of this receptor attenuates many of the behavioral effects of cocaine in rodent models (Bubar and Cunningham 2006). It is important to note that the selective antagonist used in these experiments, M10097, has already been tested in Phase III clinical trials for antipsychotic effects (Damsa et al., 2003). While it was not effective as an antipsychotic it did pass all toxicology tests. Therefore, there is good reason to believe that it is safe and tolerable as a medication. As such, the results of these experiments may have particular relevance for the treatment of MDMA abuse.

A full mechanistic understanding of 5-$HT_{2A}$ receptor modulation of dopamine neurotransmission has not been achieved. However, previous studies, using local infusion of selective antagonists, suggest that modulation of cortical glutamatergic projection neurons may be an important component of this capacity of 5-$HT_{2A}$ receptors (Pehek et al., 2001; Pehek et al., 2006). However, other work casts doubt on this mechanism as intra striatal infusion of the selective 5-$HT_{2A}$ antagonist M100907 attenuated MDMA-elicited dopamine release (Schmidt et al., 1994). In apparent contrast with these findings, intra striatal infusion of M100907 did not attenuate the locomotor-stimulant effects of systemically administered cocaine whereas local infusion into the midbrain ventral tegmental area did (McMahon et al., 2001). While a mechanistic understanding of the relationship between the 5-$HT_{2A}$ receptor and dopamine neurotransmission may not yet have been achieved, this work, collectively,

supports the contention that this receptor represents a viable target for treating MDMA abuse.

The mechanism underlying SSRI attenuation of the behavioral and neurochemical effects of MDMA is likely mediated by competition at the SERT (Gudelsky and Nash 1996; Mechan et al., 2002). In the present experiments, SSRI pretreatment attenuated the prolactin secretion elicited by S,R(+/-)-MDMA. Further experiments showed that combined treatment with a 5-$HT_{2A}$ receptor antagonist was required to attenuate R(-)-MDMA-elicited prolactin secretion. In other experiments (data not shown), antagonism of the 5-$HT_{2A}$ receptor had no effect on serotonin release by S(+)-MDMA. Collectively, this work suggests that combined treatment with both a 5-$HT_{2A}$ receptor antagonist and an SSRI may be more effective for the treatment of MDMA abuse than the use of a 5-$HT_{2A}$ receptor antagonist alone. Indeed, previous work has shown that a SSRI, a mixed D2 dopamine/ 5-$HT_{2A}$ serotonin receptor antagonist, and a non-selective 5-$HT_2$ serotonin receptor antagonist each attenuated distinct components of the subjective effects of MDMA (Liechti et al., 2001). This combined approach may be possible with a single pharmacotherapeutic as novel antidepressants, such as nefazodone and YM-992, have been developed that function as both SSRIs and 5-$HT_{2A}$ receptor antagonists (Damsa et al., 2003). Future studies should determine the effectiveness of these dual action compounds in attenuating the effects of MDMA as undeniably this approach holds great promise for reducing abuse of this illicit and deleterious compound.

| | Receptor affinity ($K_i$) | | |
|---|---|---|---|
| | **D1** | **$\alpha_{2B}$** | **5-$HT_7$** |
| **S,R(+/-)-MDMA** | 9921 | 785 | 1138 |
| **S(+)-MDMA** | 333 | 477 | NI |
| **R(-)-MDMA** | NI | 1121 | 1072 |

**Table 5-1.** Affinity ($K_i$) of MDMA and each of its stereoismers for the dopamine D1, adrenergic α2c, and serotonin 5-$HT_7$ receptors. Affinity determinations were carried out in transfected cells expressing cloned human receptors by the National Institute of Mental Health Psychoactive Drug Screening Program. Assay methods can be accessed via the NIMH-PDSP website (http://pdsp.med.unc.edu/pdspw/clones.php).

NI – inhibition of specific binding by radiolabeled ligand less than 50%.

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