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**Disruption of the Discriminative Stimulus Effects of (+)-3,
4-Methylenedioxymethamphetamine (MDMA) by (±)-MDMA
Neurotoxicity: Protection by Fluoxetine**

Thomas B. Virden III
Western Michigan University

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**DISRUPTION OF THE DISCRIMINATIVE STIMULUS EFFECTS OF
(+)-3, 4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA)
BY (±)-MDMA NEUROTOXICITY:
PROTECTION BY FLUOXETINE**

by

Thomas B. Virden III

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Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
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Western Michigan University
Kalamazoo, Michigan
June 1998

**DISRUPTION OF THE DISCRIMINATIVE STIMULUS EFFECTS OF
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It is well established that repeated, high doses of 3, 4-methylenedioxy-methamphetamine (MDMA) result in the long-term depletion of serotonin levels and destruction of serotonergic terminals in various locations in the brains of a variety of species. Further, it is also well known that concomitant injections of the serotonin reuptake inhibitor, fluoxetine, prevents this deterioration. It has recently been noted that such MDMA neurotoxicity disrupts stimulus control in rats trained to discriminate MDMA from saline in a drug discrimination procedure (Schechter, 1991a).

In order to extend Schechter's findings to the optical isomers of MDMA and to explore the potential of fluoxetine for the prevention of the disruption of the isomers' discriminative stimulus control by neurotoxicity, rats were trained to discriminate either (+)-MDMA or (-)-MDMA in a two-lever water reinforced operant procedure. Most of the rats administered (-)-MDMA died during the neurotoxic administration, obviating any conclusions thereof. However, the stimulus control by (+)-MDMA was maintained in rats administered concomitant injections of fluoxetine and the neurotoxic dose of (±)-MDMA, but disrupted in those that received (±)-MDMA with

concomitant saline injections. Control by (+)-MDMA was reestablished in these latter rats with subsequent training sessions. Postmortem neurochemical analysis verified the neurotoxic effects of the (±)-MDMA injection regimen in that serotonin and its major metabolite 5-HIAA were significantly diminished in the prefrontal cortices in rats given (±)-MDMA relative to control. Conversely, serotonin levels in rats administered concomitant (±)-MDMA and fluoxetine injections were unaffected relative to control, indicating pharmacological protection against MDMA neurotoxicity.

The deaths of the (-)-MDMA rats are discussed in light of the predominant environmental variables, and it is suggested that elevated temperatures during (±)-MDMA treatment may have contributed to their mortality. However, the results from the surviving rats indicate that the discriminative stimulus control of (+)-MDMA as disrupted by (±)-MDMA neurotoxicity can be established, regained, and protected against. Although there appears to be a relative paucity in research regarding the behavioral consequences MDMA neurotoxicity, the present findings shed new light on the potential use of fluoxetine as a tool for such explorations.

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CHAPTER I

INTRODUCTION

The ring-substituted phenylisopropylamine 3, 4-methylenedioxy-methamphetamine (MDMA, "Ecstasy") is a drug that is as singular as it is popular. Although it is an amphetamine derivative, MDMA's subjective and discriminative properties do not altogether mimic its parent drug. At high doses, this compound produces neurotoxic effects on the serotonergic system (Stone, Johnson, Hanson & Gibb, 1988). However, little is known as to the consequential behavioral effects of MDMA neurotoxicity. The present paper will attempt to shed light on this compound by focusing on its discriminative stimulus effects through a series of channels. First, a brief overview of the drug discrimination paradigm, via a short discussion of the use of drugs as discriminative stimuli, the history and general methods of the drug discrimination procedure, and a synopsis of the limitations inherent in such a paradigm will be provided. The paper will then outline the history of MDMA and discuss designer drugs in general. The subjective and behavioral effects of this enigmatic drug as well as the effects of MDMA as a discriminative stimulus will then be considered, followed by a treatment of its physiological and neurotoxic effects. The present paper will conclude with a description of an experimental study that has provided new information regarding the effects of the neurotoxic properties of MDMA and the protection thereof on the discriminative stimulus properties of the (+) enantiomer of this drug.

An Overview of the Drug Discrimination Procedure

Drugs as Discriminative Stimuli

The discriminative stimulus is widely considered a mainstay of the analysis of behavior and, as it is a primary component of the drug discrimination procedure, it seems necessary to briefly define and discuss the terms with which drugs may serve as discriminative stimuli. Michael (1982) offered a definition of the discriminative stimulus that requires the discriminative stimulus to increase the frequency of a given response by virtue of the correlation of the would-be discriminative stimulus with an increase in the probability with which that response has been followed by an effective reinforcer. Thus, to be established as a discriminative stimulus, a drug must produce subjective effects that are detectable and suitable conditions of differential reinforcement must be arranged in the drug's presence. Various drugs have been established as discriminative stimuli in many species (e.g., human, rat, gerbil, pigeon, mouse, cat, dog). For the most part, no general difference in drug discriminability has been found among these species (Lal, 1977).

Most psychoactive drugs and almost all drugs of abuse appear to exhibit discriminative control (D'Mello & Stolerman, 1978). These drugs seem to control behavior as discriminative stimuli as effectively as other, more conventional, discriminative stimuli--such as visual stimuli or even electric shock (Overton, 1964; Harris & Balster, 1971). Further, the speed of acquisition of behavior under control of

a drug as a discriminative stimulus is not significantly different from the speed of acquisition of responses controlled by other modes of discriminative stimuli (Overton, 1988). A rather long list of drugs established as discriminative stimuli includes: sedative-hypnotics, anesthetics, anxiolytics, muscarinic and nicotinic cholinergic agonists, narcotic analgesics, cholinergic agonists, dopamine receptor agonists, psychotomimetics, amphetamines, antidepressants, and--to a lesser extent-- psychotropic drugs. Some drugs, however, such as nicotine blockers, lithium, and salicylates, however, appear to exhibit little or no discriminative stimulus properties (Lal, 1977; Colpaert, 1977; Seiden & Dykstra, 1977; Overton, 1988; Kamien, Bickel, Hughes, Higgins, & Smith, 1993).

It is important to note that discriminable drugs acquire their effectiveness solely by virtue of the history of differential reinforcement, and the maintenance of the drug's discriminable properties rely on the continual application of differential reinforcement. Thus, the acquisition of discriminative stimulus properties by a drug can be explained in simple, well-established behavioral terms. No unique analyses are required.

The Drug Discrimination Procedure

During the genesis of the drug discrimination assay, the most common method of establishing differential response patterns in laboratory animals involved maze-running or similar measures (Overton, 1964). Typically, the subject was administered a drug or vehicle and placed in the start box of an electrified maze with two or more

goal boxes (e.g., a T-maze). Shock was then presented while the animal was allowed to run freely until it reached the appropriate goal box, at which point the shock was terminated. Drug administration conditions were generally alternated along with the locations of the appropriate goal box. For example, a rat trained to discriminate cocaine from saline would have been placed in a simple two-choice (right or left) electrified T-maze with shock presented. If the rat was administered cocaine, the shock would have been terminated when the rat enters the drug-appropriate (e.g., left) goal box. Conversely, if the rat was administered drug vehicle, the shock would have been terminated only when the rat entered the vehicle-appropriate (e.g., right) box. Ten trials per day would typically have been conducted with each drug in a single 10 min training session. The T-maze was preferred at this point in the development of the drug discrimination procedure, as the subjects quickly learned to appropriately and consistently turn left or right within a few trials (Overton, 1964).

One of the first studies involving the discriminative stimulus effects of drugs was performed by Culler, Coakely, Shurragar and Ades (1939), who demonstrated that different responses could be brought under control of curare as a discriminative stimulus. Conger (1951) later demonstrated that ethanol could acquire discriminative control in rats trained to run down a telescopic tunnel. The first drug generalization test was performed by Overton (1961). After discrimination training was completed, other drugs were administered in lieu of the training drug. In general, drugs previously reported to have similar effects to the training drug produced similar responses.

A modification of the T-maze procedure was implemented by Kubena and Barry (1969) who trained rats to differentially respond to drug states by their rates of responding on a lever, rather than by their choice of which extended wing of a T-maze to run . This was accomplished by reinforcing or punishing lever-pressing depending on drug presentation. That is, if the rat were administered a drug, lever presses would be followed by food presentation, however, the same lever press would be followed by shock if the rat were administered vehicle. Kubena and Barry also used a two-choice assay in which food presentation would follow a press on the appropriate lever, depending on drug (e.g., left) or vehicle (e.g., right) administration. To test for antecedent stimulus control, no food was delivered for the first 5 min of some sessions.

Lever pressing appears to be a more sensitive response for study than maze-running and is therefore more frequently used in current research. While the use of the maze only allows for a schedule of continuous reinforcement, a variety of reinforcement schedules has been used with lever-pressing. The description of the latter procedure most closely fits those most common in contemporary use. In general, the procedures of drug discrimination studies currently used share five common features: (1) subjects are trained to discriminate a given dose of a particular drug from a vehicle; (2) the reinforced responses controlled by the presence or absence of a drug are operant in nature; (3) responses are mutually exclusive; (4) these responses are similar in topography; and (5) the reinforcer presented for each

appropriate response is consistent (Colpaert, 1987). The most common dependent variable generally considered in such assays is the percentage of responding on the drug-appropriate lever prior to the first reinforcer, as well as during the overall session. Other measures, however, are also commonly considered, such as response latency and response rate as compared to vehicle conditions. These measures are also helpful in determining the maximum test dose.

In general, the criterion for successful drug-appropriate responding during discrimination training is set *a priori* at 80%-90% correct responding for the first reinforcer presentation, as well as the entire session (Colpaert, 1987). Discriminative stimulus control is considered to be established after a predetermined number of consecutive successful sessions (e.g., 9 or 10 out of 10).

Once discriminative stimulus control has been established, the generalizability of the drug's control can be determined. As suggested earlier, this is simply performed by administering a test drug in lieu of the training drug and measuring the percent correct responding on the drug-appropriate lever. This allows the researcher to "compare" a novel drug or the training drug at a novel dose to the training drug at a given dose and the vehicle condition, thus indicating the subjective similarities--or lack thereof--of the doses and/or compounds (Seiden & Dykstra, 1977). The discriminative stimulus effects of drugs and their subjective properties seem to be well correlated. For example, Callahan and Appel (1988) reported that in nonhumans trained to discriminate the hallucinogen lysergic acid diethylamide (LSD) from vehicle,

substitution occurred with psilocybin and mescaline, two drugs that are reported by humans to be rather similar to LSD. Similarly, amphetamine produces cocaine-like responding in rats trained to discriminate cocaine from saline, and vice versa (D'Mello & Stolerman, 1977). When drugs exhibit this sort of reciprocal generalization, it is often referred to as *cross generalization*.

When dose-response generalization gradients are constructed with this method, the gradients yielded are similar to those found with other forms of stimuli (e.g., tones or lights). As the test dose approaches the training dose, the corresponding patterns of response become more similar (Colpaert, 1987). When substitution tests are conducted with different drugs, the patterns of response tend to be more similar when the neuropharmacological actions of the test drug are more similar to those of the training drug (Colpaert, 1977, 1985, 1987; Lal, 1977; Overton, 1988). Such generalization tends to occur only with drugs of the same pharmacological class. That is, although response patterns engendered by heroin may generalize to morphine, they will not generalize to cocaine (Colpaert, 1987). As a result, the substitution test of the drug discrimination procedure is often used as a sensitive assay to establish the classification of novel drugs (Lal, 1977).

A putative antagonist can also be administered in conjunction with the training drug to determine the degree to which the antagonist disrupts the stimulus control maintained by the training drug. This procedure can yield information as to the neuropharmacological actions by which the training drug produces its discriminable

effects when the neuropharmacological actions of the antagonist are known. For example, if a given antagonist is known to block the effects of a specific receptor and discriminative control of the training drug deteriorates when these two drugs are co-administered, it is implied that that receptor in some manner modulates the discriminable effects of the training drug. This interpretation, however, may be confounded if the antagonist produces sensory effects of itself. The co-administration of these drugs may produce subjective effects distinct from those produced by the training drug alone, thus disrupting discriminative stimulus control regardless of the presence or absence of pharmacological antagonism.

One primary use of the drug discrimination procedure is to determine the relative discriminability of a given drug. Discriminability is often determined in one of several ways. The *sessions to criterion* (STC) measure is determined simply by calculating the number of sessions each rat was exposed to before the criterion for discriminative control was met. Thus, STC is used as an indicator of the speed of acquisition of discriminative stimulus control (Overton, 1982). Another method to determine drug discriminability is by calculating the dose at which the drug produces drug-appropriate responding in 50% of the subjects (ED_{50}) (Seiden & Dykstra, 1977). Still another index of a drug's degree of discriminability is the *asymptotic accuracy* engendered by the drug, or the relative frequency of correct responses after a protracted training period (Overton, 1988).

When the results of antagonism and substitution tests are considered in conjunction with training data, a neuropharmacological profile of a given drug can be created to describe the possible mechanisms by which the drug produces its discriminable effects (Lal, 1977). Thus, the drug discrimination procedure has been widely adopted in the field of behavioral neuroscience. In general, drug discrimination procedures are used to address three issues: (1) which drugs have discriminative stimulus properties; (2) which drugs produce generalization; and (3) which drugs antagonize the discriminative stimulus effects of other drugs (Weissman, 1977).

Limitations to Drug Discrimination

Some debate has transpired with regard to the drug discrimination procedure that merits discussion at this point. Testing may be performed either in extinction or during multiple, reinforced trials. The use of reinforced trials has the advantage in that it allows for the examination of possible effects of test treatments on overall response rate. It is commonly held, however, that testing under extinction conditions is superior in that reinforcement may provide new discriminative stimulus learning that may confound the results of the procedure (Colpaert, 1987). Further, it has been suggested that by reporting the initially selected operandum, the researcher may provide a better indication of drug-induced discriminative stimulus control than by comparing overall responding on both operandi. Comparing overall responses may be confounded by tendencies to "probe" the correct operandum as extinction takes effect. To help settle

this debate, D'Mello and Stolerman (1978) performed a series of experiments and reported a high correlation between the initially selected lever and the percentage of responding on that lever. Thus, there appears to be no advantage to limiting measures to the comparison of the initially selected lever.

Although the drug discrimination procedure yields objective and readily measurable quantification of possible subjective effects, the dependent variable must be considered as a derived variable which is designed to accommodate some previously determined theory or concept (Colpaert, 1987). The two measures most widely used to describe drug discrimination are response selection and percentage of drug appropriate responding. The latter measure, however, is frequently reported--in most studies--with no report as to why that measure was chosen over the former. Further, the percent of responses are commonly reported rather than the absolute number of drug-appropriate responses. Colpaert (1987) asserted that this practice is merely an attempt to compensate for possible drug effects on "total response output" (p 352). The measurement of total response output involves the comparison of occurrence of drug-appropriate responding as opposed to vehicle-appropriate responding and is nominal in nature. According to Colpaert, this is the only measure of variation in behavior that reflects the discriminative stimulus effects of drugs. Additionally, the statistical analysis of such nominal data for each subject is easily done, but rarely reported; as the total error rate is often 10% or less, response selection data can be selected at probability levels of 0.1, 0.05 or less (Colpaert, 1987).

When analyzing nominal data, percent generalization refers to the percentage of animals that select the drug-appropriate lever. Otherwise, generalization refers to the mean percentage of drug responding across all animals. When various doses of the training drug--or other drugs--are administered in substitution tests, generalization appears to proceed in an orderly manner from 0% to 100% drug-appropriate responding as a function of dose. Typically, when drug-appropriate responding is at or above 80%, it is considered "substitution" and when it occurs at or below 20%, it is generally regarded as a complete lack of substitution. When percent responding occurs between 20% and 80%, it is often interpreted as "partial substitution". Colpaert (1987) asserts that it indicates either a ceiling level of drug effect or that generalization occurred along a dose-response curve from 0%-100% of the level of drug effect. This outcome is rather ambiguous and difficult to interpret..

Saline appropriate responding in a generalization test is usually interpreted as reflecting an absence of the training drug's discriminative stimulus effects. However, Jarbe (1986) contends that although such responding may well indicate that the effects induced by the current stimuli were dissimilar to those of the training drug, yet it yields no information about the discriminative stimulus effects of the test compound. The inherent nature of the procedure requires the subject to respond regardless of the stimulus effects of the test compound--whether or not it is similar to either training stimulus (Seiden & Dykstra, 1977). This difficulty has been somewhat compensated for in the past via procedures in which a third choice had been made available (e.g.,

White & Holtzman, 1983; France & Woods, 1985). In a procedure such as this, the subject would be trained to respond on a third operandum with reinforcement contingent on the administration of an additional training drug which is generally dissimilar to the first.

Another considerable limitation of the drug discrimination procedure lies within the time constraints of a given study. Each subject produces relatively little information at a given session. Therefore, in order to gain data of any appreciable magnitude, many sessions must be conducted within a given experiment over a relatively extensive period of time. To help alleviate this problem, Harris, Wood, and Lal (1987) proposed a method to shorten the time it takes to train rats to discriminate a particular drug from its vehicle. This method involves one to three training sessions per day (rather than the single daily sessions commonly in current use), with at least one drug session with the provision that the drug sessions are only administered following any vehicle sessions (e.g., VVD/VD/D where V represents sessions during which vehicle was administered and D represents administration of active drug) so that no drug carryovers occurred during same-day training. However, Schechter (1988a) reported that although rats trained under this procedure acquire training criterion much faster than those who have been trained in the more traditional manner, they were not as sensitive to substitution tests. Dose-response curves determined with the "fast-trained" rats are only comparable with those of the "slow-trained" rats if the substitution sessions were preceded with vehicle training sessions on the same day.

Other attempts have been made to decrease the time invested in drug discrimination studies via the development of cumulative dosing procedures. In this procedure, an experimenter would administer a relatively low dose of the test drug, test for substitution, then administer another dose within a relatively short period of time (e.g., 5 min) to increase the dose currently "in the subject" before testing again. This process is repeated until the cumulative dose administered is presumed to be equivalent to the maximum dose to be tested for substitution in the subject. This procedure allows for the determination of a dose-response curve within a single day, drastically decreasing the time necessary to collect meaningful data. However, this procedure has not yet been fully accepted, as it has at least three inherent problems (Oveton, 1988). One main disadvantage to the cumulative dosing procedure is that the drug-blood level of a drug in a particular organism is not always predictable after a series of injections. Second, the results obtained from a given test trial within a session may or may not be independent of the results obtained during the previous test trial. Finally, a series of tests in this manner may disrupt the degree of stimulus control established under training drug conditions (Overton, 1988). However, a researcher can attempt to control for this effect by periodically administering a series of training sessions in a manner consistent with the testing regimen (e.g., VVD).

Doses must be chosen very carefully when using the drug discrimination procedure. The extent to which a given compound mimics a training drug is dependent not only on the dose of the test drug, but the dose of the training drug as

well (Stolerman & D'Mello, 1981; White & Appel, 1982b). In order to substitute for a training drug at a relatively high dose, a high dose of the test compound is generally required (Overton, 1971; Stolerman & D'Mello, 1981).

It has been commonly understood that the drug discrimination procedure is helpful in classifying drugs, especially those suspected of high abuse potential. However, it is as yet unknown if the discriminative stimulus effects of an abused drug are in any way related to the subjective effects that appear to contribute to drug abuse. Unfortunately, little attention has been directed to the effects contributing to drug discrimination with respect to their possible contribution to drug abuse. Although the drug discrimination procedure has been determined to be quite helpful, it is quite limited in this regard by its very nature in that there are no other procedures available with which to measure its reliability and validity.

Although the drug discrimination procedure appears to have some limitations that are difficult to overcome, it remains highly regarded as a powerful tool for the designation of drug categories (Overton, 1984), and for the assessment of neural mechanisms underlying the stimulus effects of various psychoactive agents (Appel & Cunningham, 1986). Its widespread acceptance as a research tool is reflected by its use in over 2,500 experiments published between 1951 and 1996 (Stolerman, 1998). Through these and similar procedures, MDMA has been established as having definite discriminative stimulus properties and has since become a subject of study in a great many drug discrimination investigations.

An Overview of MDMA

A Brief History of MDMA

Methylenedioxymethamphetamine (MDMA, Figure 1) first appeared in 1912 when it was synthesized by the Merck Pharmaceutical Company in Darmstadt, Germany, by scientists searching for an appetite suppressant for soldiers during the First World War (Redhead, 1993; Beck & Rosenbaum, 1994). Patented in 1914 (Henry, Jeffreys, and Darley, 1992), this drug was virtually forgotten for nearly forty years. MDMA briefly resurfaced in 1953, when the US Army Medical Center began experimenting with the drug's anorectic properties (Redhead, 1993), but was not used recreationally until the late 1960's, when "it seems that almost anything was tried for it's psychedelic effects, e.g., smoking banana peels, sniffing Italian red peppers, etc." (Mack, 1985, p. 641). The effects of this psychedelic amphetamine was of particular interest to "new age seekers" who used the drug to induce feelings of human interconnectedness and well being (McDowell & Kleber, 1994). Thus, despite its early beginnings, MDMA has only been "on the street" for about thirty years.

When the results of the Army's experimentation were made public in the early 1970's interest in the drug began to wane but, in 1976, a small group of therapists began prescribing MDMA as an adjunct to induce communication, acceptance, and facilitate "self-examination" (McDowell & Kleber, 1994). However, the members of this group were hesitant to publish any of their results in fear that public knowledge of

the drug's use might quicken illegalization and thus prevent any future research. In 1978, however, Shulgin and Nichols published the first pharmacological account of MDMA, in which they described the drug's ability to evoke an easily controlled "state of consciousness" characterized by enhanced self-awareness, affect, and communication. These effects have since been widely reported among users (Anderson, Braun, Braun, Nichols & Shulgin, 1978; Climko, Roehrich, Sweeney, & Al-Razi, 1987; Peroutka, Newman, & Harris, 1988). Despite this publication, the potential therapeutic use of MDMA was largely unknown to the general public and its use was confined to a small population of recreational users (Beck & Rosenbaum, 1994). The recreational use of MDMA, however, was to see a dramatic increase in the next few years.

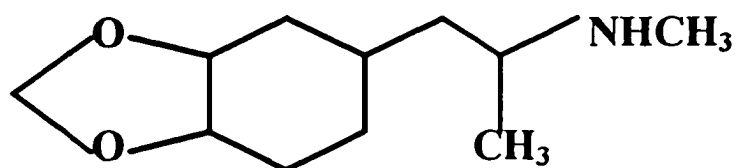


Figure 1. The Chemical Structure of MDMA.

The extra medicinal use of MDMA began to climb in the early 1980's, probably due to the efforts of some entrepreneurs from Boston known, appropriately enough, as the "Boston Group". Through mass-production and distribution by this new group of businessmen, the popularity of MDMA as a recreational drug began to rise (Beck & Rosenbaum, 1994). As the demand for the drug increased, so did the

opportunity for profit. This opportunity was seized upon by the southwest distributor for the Boston Group who, with financing from friends in Texas, began his own business. By using such promotional techniques as sale at commercial stores and bars, as well as promoting "ecstasy parties" featuring a new drug that's "fun" and "good to dance to", the Texas Group's efforts resulted in an all-time high of MDMA use (Beck & Rosenbaum, 1994). In 1985, an estimated 35 to 200 physicians used MDMA in practice and non medical use reached an approximate 30,000 doses per month prior to its illegalization (American Medical News, 1985). Since MDMA has been reported to induce feelings of connection and a certain psychomotor agitation that could be "pleasurably relieved" by dancing, its popularity in the dance clubs seemed natural. The drug became the status quo at all-night dance parties called *raves*, which have only recently gained popularity in the United States, but have been a favorite in England since the early 1980's (McDowell & Kleber, 1994). The drug had, however, been illegal in England since 1977 under the Misuse of Drugs Act of 1971 (Redhead, 1993). Unfortunately, this form of popularity would quickly take a decidedly dark turn.

A number of MDMA-related deaths began to emerge in the raves of England, apparently due to the conditions of the clubs themselves. It had been argued that loud noise, elevated temperature, (Randall, 1992) and dehydration (Green, Cross, & Goodwin, 1995) may exacerbate the toxic effects of MDMA. Thus, the most preferred place for MDMA use was also its most dangerous. It briefly became the

custom of some rather machiavellian club owners to turn off their water supply while hosting the raves. In this manner, they could enjoy enhanced profits by selling bottled water. On a particularly hot day in 1992, about 15 deaths were correlated with this practice. The British National Poisons Unit determined that the deaths were the result of a form of heat stroke, brought on by MDMA use under conditions of dehydration and vigorous dancing. The English government quickly mandated a continuous supply of water at all clubs and the number of MDMA-related deaths, coincidentally, dramatically decreased (McDowell & Kleber, 1994).

Although the Drug Enforcement Agency (DEA) of the United States had been aware of MDMA since the early 1970's, reports of its use had been entirely too infrequent to warrant investigation (Beck & Rosenbaum, 1994). In 1982, a DEA spokesperson was quoted as saying "If we can get enough evidence to be sure there's potential for abuse, we'll ban it." (Dye, 1982, p. 8). The increased popularity brought on by the Texas Group's efforts provided the DEA with the fuel it needed to criminalize MDMA. Due to its frequent use, the DEA requested in July of 1984 that MDMA be placed on Schedule I of the Controlled Substances Act (CSA), a classification that categorizes a drug as having high abuse potential and no approved medical use. This was soon supported by a DEA chemist's contention that MDMA demonstrates high abuse potential due to its widespread illicit sale and use, its clandestine manufacture, and its chemical and pharmacological similarity to amphetamine, another--already illegal--drug of abuse (Beck & Rosenbaum, 1994).

The Texas Group responded by attempting to squeeze every bit of profit possible from their product while it was still legal. During this period, the production and sale of MDMA reached its peak and in the final two months or so prior to its illegalization, a reported amount of over two million tablets were sold. This group of salesmen began selling their product at dramatically reduced rates, creating a sort of "going out of business sale". Prices were said to have reduced in some areas from \$25 to \$8 per tablet (Beck & Rosenbaum, 1994). Although the hearings to decide whether to criminalize MDMA were scheduled to take place in the Fall of 1985, the rampant sale of the drug and blatant promotion at clubs and bars (Beck & Rosenbaum, 1994) provoked Senator Lloyd Bensten (McDowell & Kleber, 1994) to petition the DEA to place MDMA on Schedule I on an emergency basis under the Comprehensive Crime Control Act of 1984 (Beck & Rosenbaum, 1994). On July 1, 1985, MDMA was temporarily placed on Schedule I of the CSA, although a substantial number of people, including therapists and the clergy, were in support of a less restrictive category (much to the surprise of the DEA) (McDowell, & Kleber, 1994).

Of course, criminalization did not eliminate the use of MDMA. A 1987 survey of college students reported that 39% still used the drug (Peroutka, 1987). If anything, it piqued the public's interest. After the July 1 scheduling, an explosion of coverage of the drug appeared in the lay press (e.g., Dowling, 1985; Gerts, 1985; Rolbein, 1985; Toufexis, 1985) and a new drug appeared. An offshoot of the Texas Group created an analog to MDMA, 3, 4-methylenedioxymethamphetamine (MDE)

and tried to market it under the name "Eve", although the strategy was largely unsuccessful, it prompted congress to pass the Controlled Substances Analog Act (CSAA) in 1986 (Beck & Rosenbaum, 1986), making the creation and sale of designer drugs illegal.

In England, the popularity of the drug carried on. In 1991, a recent study indicated that few inquiries had been made into the England's National Poisons Information Service (NPIS) in London until the second half of that year. This seems of little importance to the present issue, but when a sharp increase in inquiry frequency was noted, it piqued the researcher's curiosity. Interestingly, an increase in lab detection of MDMA was noted which correlated nicely with the increase in NPIS inquiries (Henry, Jeffreys, & Dawling, 1992).

Since amphetamine, the parent drug of MDMA, was not illegal at the time of MDMA's synthesis, it may be argued that MDMA is not a designer drug in the strictest definition of the term. Nonetheless, it is considered to be one of the most popular and well known "members" of the designer drug class. Thus, a brief discussion of the more salient characteristics of designer drugs is warranted.

Designer Drugs: A General Discussion

A designer drug is a slight variation of a drug that is already federally controlled, and designed to imitate the effects of the drug from which it was derived. Each time a new drug was discovered, law enforcement agencies were required to go

through a variety of lengthy beaurocratic procedures to have it certified as a controlled substance (Maisto, Galizio, & Conners, 1991). Thus, prior to the enactment of the CSAA, drug dealers could take advantage of this lapse by rapidly creating and distributing analogs of already illegal drugs. Since it is the parent compound that is illegal, not the analog itself, the drug distributors could sell essentially legal drugs (Evanko, 1991) and, with rapid rates of synthesis and large-scale distribution, turn very impressive profits. This practice, however, had some rather serious consequences.

Hurried mass-production and low standards of quality led the distributors to sell drugs without controlled testing or quality control. It was often unknown what behavioral or physiological effects a designer drug would have until it was distributed. The risks of introducing drugs contaminated with toxins were also then dramatically increased (Carroll, 1993). To illustrate, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) was distributed as a synthetic opiate and was found to have caused significant damage to the substantia nigra in users, resulting in parkinsonian-like symptoms (Langston, 1985; Ballard, Tetrud, & Langston, 1985; Kirsch, 1986). Controlling the production of designer drugs seemed insurmountable. By the time one drug was criminalized, another legal one had taken its place. The CSAA, which allows for the immediate classification of a novel drug as a controlled substance, was seen as a solution to this problem (Maisto, Galizio, & Conners, 1991).

Unfortunately, a lapse still exists between the creation of a designer drug and its criminalization due to the lack of sensitive assays in the agencies' possession to detect novel compounds (Payne, Hahn, & Pinger, 1991). This "grace period" combined with the relative affordability and increased potency makes the continued use of designer drugs likely (Carroll, 1993). Indeed, a designer drug can be 10 to 1,000 times more potent than its parent compound (Maisto, Galizio, & Conners, 1991). For example, alpha-methyl-fentanyl is 200 times stronger than its parent compound, morphine (Beebe & Walley, 1991).

Synthetic opiates, arylhexylamines, and phenylethylamines are three of the most common categories of designer drugs. An exception, it seems, is crack, a form of cocaine. Although it does not fall into any of the three categories above and has been illegal from the outset, it has been referred to as a designer drug due to its popularity and availability (Beebe & Walley, 1991). This discrepancy illustrates the relatively relaxed definition the public appears to attach to the term "designer drug". MDMA, a phenylethylamine, is a synthetic amphetamine derivative with both hallucinogenic and stimulant properties (Callahan & Appel, 1988; Evans & Johanson, 1986; Schechter, 1986a).

The Subjective and Behavioral Effects of MDMA

The most salient effects of MDMA in humans are subjective in nature. MDMA users have reported an increase in affect and self-esteem, as well as enhanced

communication and intimacy (Greer & Strassman, 1985; Schechter, 1986; Climko, Roehrich, Sweeney, & Al-Razi, 1987; Lamb & Griffiths, 1987; Peroutka, Newman, & Harris, 1988). Although a heightened sense of touch and mild visual hallucinations have been reported by MDMA users (Lamb & Griffiths, 1987), the most commonly reported effects of MDMA seem to be without the sensory distortion or dissociation from oneself that is commonly associated with hallucinogens (Greer & Strassman, 1985; Schechter, 1986; Climko et al., 1987; Peroutka et al., 1988). This drug was likely used as a psychotherapeutic adjunct due to its reported ability to enhance feelings of interconnectedness and empathy (Downing, 1986; Grinspoon & Bakalar, 1986).

The subjective effects of MDMA appear to occur in three phases in humans. The first 30-minute interval is often referred to as "The Weird Period" (WP). The WP is often followed by a "rush" of "tingling sensations" and finally a "high" that lasts from 3 to 5-hours (Schechter, 1987). After the 3 to 5-hour high, the effects are largely dissipated except for a mild residual sympathomimetic stimulation that can last for several hours (Schechter, 1987). The subjective effects of withdrawal include muscle aches, depression, drowsiness, and a deficit of concentration (Barnes, 1988).

The study of nonhuman subjects has facilitated the controlled observation of behaviors affected by MDMA. Fortunately for many food-reinforced studies, MDMA seems to have little, if any, anorectic effects as would be shown by the subjects' lack of willingness to respond for food (Boja & Schechter, 1987). The behavioral effects of

MDMA are most commonly compared to those of amphetamine. MDMA has been noted to be self administered by rhesus monkeys and baboons (Beardsley, Balster & Harris 1986; Lamb & Griffiths, 1986), to increase self-stimulation of the brain (Hubner, Bird, Rassnick, & Kornetsky, 1988), and decrease prepulse inhibition in a manner similar to such psychotomimetics as apomorphine and amphetamine (Mansbach, Braff, & Geyer, 1989). Like amphetamine, the drug increases startle amplitude (Mansbach et al., 1989). MDMA seems to produce an increase in locomotor stimulation that is similar to that seen with amphetamine (Braun, Shulgin, & Braun, 1980; Gold & Koob, 1980), but at doses that do not induce stereotypy (Gold, Koob, & Geyer, 1988). The MDMA-induced increase of locomotor activity seems to have a more prolonged duration than those of amphetamine and a time course that seems to coincide with the subjective effects reported by humans (Shulgin & Nichols, 1978; Beck & Morgan, 1986). This drug has also been shown to be effective in producing conditioned taste aversion, but is not as potent in this capacity as amphetamine (Lin, Atrens, Christie, Jackson, & McGregor, 1993). Similar to cocaine and amphetamine, MDMA has been demonstrated to produce conditioned locomotor activity accomplished by consistent pairings of drug administration and salient environmental cues (e.g., odor) (Gold & Koob, 1989). Also similar to these classical psychostimulants, MDMA has also been demonstrated to produce conditioned place preference (Bilsky, Hui, Hubbel, & Reid, 1990; Schechter, 1991). Interestingly, a

neurotoxic dose regimen (20 mg/kg, delivered s.c. twice daily for 4 days) does not change this conditioning (Schechter, 1991).

The effects of MDMA on schedule-controlled behavior have been explored a little more sparsely. Acute administrations of MDMA have been shown to decrease responses in a dose-dependent manner in: mice maintained under a FR 20 schedule of food delivery (Glennon, Little, Rosencrans, & Yousif, 1987); pigeons under both components of a multiple FR 30 FI 3-min schedule of food delivery (Nader, Hoffman, & Barrett, 1989); and monkeys under a repeated acquisition procedure (Thompson, Winsauer & Mastropaolo, 1987). Frederick, Gillam, Allen and Paule (1995) demonstrated that acute MDMA administration reduced performance accuracy in monkeys under temporal response differentiation (TRD) and incremental repeated acquisition (IRA) conditions. Drug administration also lowered the break point under a progressive ratio (PR) schedule of food delivery. In each of these conditions, response rate was not affected and no effect was found on performance under delayed-matching-to-sample (DMTS) or conditioned position responding (CPR) procedures (Fredrick, Gillam, Allen & Paule, 1995). However, in a different study, acute administration of MDMA induced lower performance accuracy in monkeys under a CPR schedule (Frederick, Ali, Slikker, Gillam, Allen, & Paule, 1995).

Relatively few studies have examined the chronic effects of MDMA administration on behavior. Zancy, Virus, and Woolverton (1989) demonstrated that when MDMA was administered chronically to mice, milk drinking initially decreased,

but behavioral tolerance eventually developed. In rats under an interresponse-time-greater-than-72-s ($IRT > 72$ -s) schedule, MDMA initially decreased response rates and reduced reinforcement rates. After chronic administration, sensitization occurred and the effects of MDMA were more pronounced (Li, Marek, Vosmer, & Seiden, 1989). In monkeys, chronic drug administration produced tolerance to the MDMA-induced disruption of accuracy under TRD, CPR, and IRA schedules (Frederick, Ali, Slikker, Gillam, Allen, & Paule). Finally, MDMA administration decreased the overall response rate and accuracy of pigeons under a DMTS schedule (LeSage, Clark, & Poling, 1993). Similar to Frederick et al. (1995), although no deterioration of performance was noted, tolerance to the acute effects of MDMA developed after chronic administration (LeSage, Clark, & Poling, 1993).

Although it has been repeatedly stated that the behavioral effects of MDMA under a variety of conditions have been studied with inadequate detail, the ability of MDMA to serve as a discriminative stimulus appears to be an exception to this contention. Since MDMA has distinct subjective, physiological, and behavioral effects that have been demonstrated to be similar to psychostimulant and hallucinogenic drugs, it seems intuitive that MDMA should be able to serve as a discriminative stimulus or substitute for other drugs that have previously been established as discriminative stimuli. Callahan & Appel (1987) reported that although LSD and mescaline are both hallucinogens, the discriminative stimulus effects of MDMA are more similar to mescaline than LSD. At certain doses, MDMA has been shown to

produce discriminative stimulus effects similar to amphetamine in pigeons (Evans & Johnson, 1986), rats (Glennon and Young 1984), and monkeys trained to discriminate amphetamine from saline (Kamien, Johanson, Schuster, & Woolverton, 1986).

However, amphetamine seems to have rather inconsistent effects in rats. Oberlender and Nichols (1988) demonstrated that amphetamine substituted for MDMA in rats, but Schechter (1989) failed to replicate this finding. Thus, it appears that the discriminable effects of MDMA are far from completely understood.

The Discriminative Effects of MDMA

The first report that MDMA possessed stimulus discriminative control properties came via an abstract by Glennon, Titeler, Lyon, and Yousif (1986). The first detailed description, however, was made by Schechter (1987) who trained rats to discriminate (\pm)-MDMA from saline in a two-lever procedure using an FR 10 schedule of food delivery. Schechter reported that (\pm)-MDMA substituted for itself in a dose-dependent manner, as did its optical isomers. The R(-) enantiomer, however, was shown to be slightly less potent than the S(+) in this regard. Although (+)-MDMA has been reported to be more active than the racemate (Anderson, Bronson, Braun, Nichols, & Shulgin, 1978), (\pm)-MDMA is a more potent substitute for itself than either isomer (Schechter, 1987). This finding is supported by in vitro findings by Nichols, Lloyd, Hoffman, Nichols, & Yim (1982), which demonstrated that (\pm)-MDMA was more potent in promoting monoamine release than either isomer. The discriminative

stimulus properties of MDMA seem to have a time-course of 10 to 90 min and rats demonstrate 100% drug-appropriate lever-pressing when tested at 20 and 60 min following MDMA administration (Schechter, 1987). The (-) isomer of MDMA also appears to have a longer time-course than does the (+) isomer (Baker, Virden, Miller, & Sullivan, 1997).

The results of drug discrimination studies using (±)-MDMA as a training drug are summarized in Table 1. These findings appear to correlate well with some subjective reports in humans. For example, the subjective effects of 3, 4-methylenedioxyamphetamine (MDA), a major metabolite of MDMA (Fitzgerald, Blanke, Narisamhachari, Glennon, & Rosencrans, 1987) and a recreational drug of lesser popularity (Cho, Hiramatsu, Distefano, Chang, & Jenden, 1990; Yousif, Fitzgerald, Narasimhachari, Rosencrans, Blanke, & Glennon, 1990), seem to resemble the subjective effects of MDMA in humans (Shulgin & Nichols, 1978). Likewise, MDMA has been shown to substitute in nonhuman subjects trained to discriminate MDA (Glennon & Young, 1984) or its isomers (Broadbent, Appel, Michael, & Ricker, 1992) from vehicle. Racemic MDMA also substitutes for its N-ethyl derivative N-ethyl-3, 4-methylenedioxyamphetamine (MDE; Eve) (Boja & Schechter, 1987) and produces stimulus generalization to N-methyl-1-(1, 3-benzoioxyl-5-yl)-2-butanamine(MBDB), the alpha-ethyl homologue of MDMA (Oberlender & Nichols, 1988). MDE also substitutes for (±)-MDMA as does N-hydroxy MDA (N-OH-

Table 1

Results of Substitution Tests in Drug Discrimination Procedures
Using (\pm)-MDMA as a Training Drug

Drug	Substitution? (Y/N/??)	Source
MDE	Y	Glennon & Meisenheimer (1989)
N-OH-MDA	Y	Glennon & Meisenheimer (1989)
3-OH-PMMA	N	Glennon & Higgs (1992)
3, 4-DMA	N	Glennon & Higgs (1992)
N-Me-3, 4-DMA	N	Glennon & Higgs (1992)
PMA	N	Glennon & Higgs (1992)
PMMA	Y	Glennon & Higgs (1992)
LSD	Y	Oberlender & Nichols (1988)
DOM	N	Oberlender & Nichols (1988)
ibogaine	N	Schechter & Gordon (1993)
amphetamine	Y	Oberlender & Nichols (1988)
	??	N Schechter (1989)
fenfluramine	Y	Baker et al. (1995); Schechter (1986a, 1989)
norfenfluramine	Y	Schechter (1989)
TFMPP	Y	Schechter (1989)
PCA	Y	Schechter (1986a)
THBC	Y	Schechter (1986b)
MMAI	Y	Marona-Lewicka & Nichols (1994); Huang et al. (1992)
8-OH-DPAT	N	Glennon (1987a)
DOI	N	Glennon (1987a)
PIA	Y	Fuller et al. (1980)
5-iodo-2-aminodan	Y	Nichols et al. (1991)
MTA	Y	Johanson et al. (1991)
apomorphine	N	Young & Glennon (1986)
l-cathinone	N	Young & Glennon (1986)

MDA), another structural analogue of MDMA (Glennon & Meisenheimer, 1989).

Although MDMA, MDE, and N-OH MDA have similar subjective effects, N-OH MDA appears to be slightly more potent than MDMA, and MDE is slightly less potent (Braun, Shulgin, & Braun, 1980). These findings illustrate the similarities between the results indicated from drug discrimination studies and human reports of MDMA's subjective effects.

In an attempt to determine whether metabolite-related analogues of MDMA produce stimulus effects similar to those of their parent compound, Glennon and Higgs (1992) performed substitution tests with several MDMA analogues in rats trained to discriminate (\pm)-MDMA from saline. The five analogues tested were: N-monomethyl-1-(3-hydroxy-4-methoxyphenyl)-2-aminopropane (3-OH-PMMA); 1-(3, 4-dimethoxyphenyl)-2-aminopropane (3, 4-DMA); N-Me 3, 4-DMA; PMA; and N-methyl-1-(4-methoxyphenyl)-2-aminopropane (PMMA). Only PMMA produced (\pm)-MDMA-like responding.

Attempts to classify the discriminative stimulus effects of MDMA, however, have yielded mixed results. Table 2 summarizes the results of experiments attempting to delineate the discriminative effects of the optical isomers of MDMA. Although MDMA has been reported to have hallucinogenic effects and affect the serotonin (5-HT) neuronal systems, neither isomer of MDMA has been shown to substitute for the hallucinogens 2, 3-dimethoxy-4-methylisopropylamine (DOM) (Glennon, Young,

Table 2

Results of Substitution Tests in Drug Discrimination Procedures Using
(+)-MDMA or (-)-MDMA as a Substitution or Training Drug

Procedure	Drug	Substitution?	Source
(+) -MDMA as training drug	mescaline	N	Baker et al. (1995)
	LSD	N	Baker et al. (1995)
	cocaine	N	Baker et al. (1995)
	amphetamine	N	Baker et al. (1995)
(-) -MDMA as training drug	mescaline	N	Baker et al. (1995)
	LSD	Y	Baker et al. (1995)
	cocaine	N	Baker et al. (1995)
	amphetamine	N	Baker et al. (1995)
(+) -MDMA as substitution drug	DOM	N	Glennon et al. (1982)
	α -MeT	N	Glennon (1993)
	LSD	N	Callahan & Appel (1988)
	mescaline	Y	Callahan & Appel (1988)
	amphetamine	Y	Glennon et al. (1988)
		??	N Oberlender & Nichols (1988)
(-) -MDMA as substitution drug	DOM	N	Glennon et al. (1982)
	α -MeT	N	Glennon (1993)
	LSD	N	Callahan & Appel (1988)
	mescaline	Y	Callahan & Appel (1988)
	amphetamine	N	Oberlender & Nichols (1988)

Rosencrans, & Anderson, 1982), α -methyltryptamine (α -MeT) (Glennon, 1993) or LSD (Callahan & Appel, 1988). However, both isomers were found to substitute for the hallucinogen mescaline (Callahan & Appel, 1988) and the racemate generalized to the α -ethyl homologues of both DOM and α -MeT (Glennon, 1993). Additionally, LSD was found to substitute for (\pm)-MDMA, but DOM did not (Oberlender & Nichols, 1988). Yet, another study indicated that mescaline did not substitute for either isomer of MDMA, and LSD only substituted for (-)-MDMA (Baker, Broadbent, Michael, Matthews, Metosh, Saunders, West, & Appel, 1995). Further, Schechter and Gordon (1993) report that ibogaine, a drug that produces both stimulant (Gershon & Lang, 1962) and hallucinogenic (Clineschmidt, Zacchel, Totaro, Pflueger, McGruffin, & Wishoutsky, 1978) effects in humans, demonstrated no stimulus generalization to (\pm)-MDMA. It is also interesting to note that, despite the similarities of discriminative stimulus control found between (+)-MDA and cocaine (Broadbent, Michael, & Appel, 1989; Broadbent, Appel, Michael, & Ricker, 1992), this CNS stimulant was found to substitute for neither isomer of MDMA (Baker, Broadbent, Michael, Matthews, Metosh, Saunders, West, & Appel, 1995). It is also rather interesting to point out that in rats trained to discriminate Δ^9 -tetrahydrocannabinol (THC), (\pm)-MDMA produced no drug-appropriate responding (Barrett, Wiley, Baslter, & Martin, 1995).

Table 3 summarizes the results of investigations of the effects of (\pm)-MDMA as a substitution drug in rats trained to discriminate a variety of psychoactive drugs.

Although (+)-MDMA was reported to substitute for amphetamine in one study (Glennon, Yousif, & Patrick, 1988) other researchers reported no substitution with either isomer (Oberlender & Nichols, 1988) or the racemate (Oberlender & Nichols, 1988; Glennon & Meisenheimer, 1989). However, as previously stated, racemic MDMA was found to substitute for amphetamine in studies using rats (Glennon &

Table 3
Results of Substitution Tests in Drug Discrimination Procedures
Using (\pm)-MDMA as a Substitution Drug

Drug	Substitution?	Source
MDA	Y	Glennon & Young (1984)
(+)-MDA	Y	Broadbent et al. (1992)
(-)-MDA	Y	Broadbent et al. (1992)
MDE	Y	Boja & Schechter (1987)
MBDB	Y	Oberlender & Nichols (1988)
α -ethyl homologue of DOM	Y	Glennon (1993)
α -ethyl homologue of α -MeT	Y	Glennon (1993)
amphetamine	??	Y Glennon & Young (1984); Evans & Johanson (1986); Kamien et al. (1986) N Oberlender & Nichols (1988); Glennon & Meisenheimer (1989)
methamphetamine	N	Glennon & Higgs (1992)
fenfluramine	Y	Schechter (1986a, 1989)
lisuride	N	White & Appel (1982a)
bromocriptine	N	Holohean et al. (1982)
TFMPP	N	Schechter (1988b)
l-cathinone	Y	Schechter (1986a)
THC	N	Barret et al. (1995)

Young, 1984), pigeons (Evans & Johanson, 1986), and monkeys (Kamien, Johanson, Schuster, & Woolverton, 1986). Further, Oberlender and Nichols (1988) demonstrated that (+)-amphetamine substitutes for (±)-MDMA. However, Schechter (1989) failed to replicate this finding. Glennon and Higgs (1992), interestingly, reported that MDMA did not produce stimulus generalization to methamphetamine. When a drug is found to substitute for another compound, but is not substituted by the compound when the initial test drug is used as a training drug, the substitution is referred to as *asymmetrical*. Although amphetamine and (±)-MDMA yield contradictory results, findings have been more consistent regarding the 5-HT releasers such as the amphetamine derivative fenfluramine, and its major metabolite norfenfluramine (Schechter 1986a, 1989).

Although fenfluramine is structurally similar to amphetamine and both have been used therapeutically as anorectics (Evans, Zancy, & Johanson, 1990), some significant differences may account for this apparent lack of discriminative similarity with regards to MDMA substitution: Amphetamine facilitates the release of the catecholamines, particularly dopamine (DA). (Glowinski, 1970; Leibowitz, 1978). In contrast, fenfluramine facilitates 5-HT release (Garattini, Barroni, Mennini, & Samanin, 1981; Jespersen & Scheel-Kruger, 1973). Interestingly, amphetamine has been found to be freely self-administered in monkeys (Balster & Schuster, 1973) and in humans (Johanson & Uhlenthuth, 1982), but this has not been demonstrated with fenfluramine (Johanson & Uhlenthuth, 1982; Woods & Tessel, 1974). Further, where

fenfluramine is found to produce non-stimulant-like subjective effects in humans (Chait, Uhlenhuth, & Johanson, 1986; Gotesdam & Gunner, 1972; Griffith, Nutt, & Jasinski, 1975) and sedative effects in rats (Ziance, Sipes, Kinnard, & Buckley, 1972), amphetamine increases rodent locomotor activity (Cox & Maickel, 1972) and produces stimulant-like subjective effects in humans (Chait, Uhlenhuth, & Johanson, 1986).

Inconsistencies such as the asymmetrical cross-substitution of MDMA and amphetamine also appear to indicate that the extent to which the discriminative stimulus effects of MDMA are amphetamine- or hallucinogen-like may depend on the training drug and the procedures used. Schechter (1989), for example, utilized an FR 10 food-reinforced schedule while Oberlender and Nichols (1988) used an FR 50 schedule and the dose at which amphetamine substituted for (\pm)-MDMA disrupted responding in nearly half of the subjects. Glennon and Meisenheimer (1989), on the other hand, utilized a VI 15 schedule of sweetened powdered milk delivery during 15 min sessions held 15 min after injection. In the study by Evans & Johanson (1986), pigeons were trained on a 3-key FR 30 food delivery schedule to discriminate fenfluramine, amphetamine, and saline vehicle from one another. When administered (\pm)-MDMA, two of the three pigeons responded on the amphetamine-appropriate key, and the other responded on the key previously active after fenfluramine administration. Further, Stolerman and D'Mello (1981) suggest that drug-produced stimulus control in general may be mediated by multiple drug effects. Compounds without partially

overlapping profiles of action may have to be administered in relatively larger doses to provide shared effects with an intensity adequate for stimulus generalization. Perhaps it is the case that MDMA and amphetamine have profiles of action which overlap to the extent that some generalization may occur, but differ significantly with respect to their primary pharmacological activities (Oberlender & Nichols, 1988). The degree to which these factors may influence the discriminative stimulus effects of a given drug has not heretofore been determined.

Several attempts have been made to assess the neural mechanisms of the discriminable effects of MDMA via the drug discrimination procedure. The indirect 5-HT releaser, norfenfluramine, the 5-HT releaser and putatively specific 5-HT_{1B} agonist, N-3-trifluoromethylphenylpiperazine (TFMPP) (Schechter, 1989), and the 5-HT releasers p-chloramphetamine (PCA) and fenfluramine have been shown to substitute for (±)-MDMA (Schechter, 1986a) or its isomers (Baker et al., 1995). Tetra-hydro-b-carboline (THBC), which has been shown to be active in 5-HT systems, has also been shown to substitute for MDMA (Schechter, 1986b), as did the selective 5-HT releasing agent 5-methoxy-6-methyl-2-aminodan (MMAI) (Marona-Lewicka & Nichols, 1994). However, the 5-HT_{1A} agonist 8-OH DPAT and the 5-HT₂ agonist DOI have been shown to not substitute for (±)-MDMA (Glennon, 1987a), nor does (±)-MDMA substitute for lisuride (White & Appel, 1982a) or bromocriptine (Holohean, White, & Appel, 1982). Although TFMPP substitutes for (±)-MDMA, this relationship appears asymmetrical in that TFMPP yielded no stimulus

generalization to (\pm)-MDMA (Schechter, 1988b). However, the discriminative stimulus effects of MDMA and each isomer have also been shown to be attenuated by the 5-HT₂ receptor antagonist pirenpirone (Schechter, 1989; Baker, et al., 1997). Further, p-idoamphetamine (PIA), a 5-HT neurotoxic halogenated amphetamine which inhibits [¹⁴C]-5-HT uptake and blocks the degeneration of 5-HT by mitochondrial monoamine oxidase (Fuller, Snoody, Snoody, Hemrick, Wong, & Malloy, 1980), and its non neurotoxic analogue, 5-iodo-2-aminodan, have both been demonstrated to substitute for (\pm)-MDMA (Nichols, Johanson & Oberlender, 1991), as has 5-methoxy-6-methyl-2-aminoindan (MMAI), which possesses a high level of 5-HT activity and virtually no DA activity (Huang, Marona-Lewicka, & Nichols, 1992). p-Methylthioamphetamine (MTA), a potent 5-HT releaser has also been demonstrated to substitute for (\pm)-MDMA (Johanson, Frescas, Oberlender, & Nichols, 1991). These findings suggest a serotonergic involvement in the discriminative stimulus effects of MDMA. In another study, however, pirenperone has been shown not to block the discriminative stimulus effects of (\pm)-MDMA in mice (Rosencrans & Glennon, 1987).

Table 4 provides a synopsis of similar research that explored the ability of a variety of compounds to attenuate drug appropriate responding during training-dose substitution tests in subjects trained to discriminate (\pm)-MDMA from saline. The less selective 5-HT antagonist metergoline was also found to have no effect on the discriminative stimulus properties of either isomer of MDMA (Baker et al., 1995). However, the 5-HT₃ antagonists LY27854 and zacopride have been shown to block

(±)-MDMA discrimination (Glennon, Higgs, Young, Issa, 1992). Although (+)-MDMA has been shown to have some dopaminergic properties, the DA antagonists haloperidol (Young & Glennon, 1986) and CGS10746B (Altar, Wesley, Liebman, Gerhard, Kim, Welsh, & Wood, 1987) were shown not to block (±)-MDMA discrimination. Similar to some of the results found with amphetamine, the direct DA agonist, apomorphine, and the indirect DA agonist, l-cathinone, were shown not to substitute for (±)-MDMA, (Young & Glennon, 1986), but (±)-MDMA substituted for l-cathinone (Schechter, 1986a).

Table 4
Results of Antagonism Tests in Drug Discrimination Procedures
Using (±)-MDMA as a Training Drug

Drug	Substitution?	Source
pirenperone	??	Y Schechter (1989)
		N Rosencrans & Glennon (1987)
metergoline	N	Baker et al. (1995)
LY27854	Y	Glennon et al. (1992)
zacopride	Y	Glennon et al. (1992)
haloperidol	N	Young & Glennon (1986)
CGS10746B	N	Altar et al. (1987)

This seemingly exhaustive inventory of findings underscores the difficulty in establishing conclusions in reference to the neural mechanisms mediating the

discriminative stimulus effects of MDMA. It is likely to be the case that, although (-)-MDMA appears to be more serotonergic than (+)-MDMA, the discriminative stimulus effects of (±)-MDMA comprise a complex cue involving both dopaminergic and serotonergic mechanisms, thereby resulting in asymmetrical substitution and partial antagonism at best from the more selective dopaminergic or serotonergic compounds. MDMA, therefore, produces a stimulus effect that is similar to, but more complex than either hallucinogens or stimulants (Evans, Zancy, & Johanson, 1990). Wood, Lal, Yaden, and Emmett-Oglesby (1985) suggest that the subjects discriminate drug effects based on the most salient component of the training discriminative stimulus. Subjects trained to discriminate a more selective component of this complex cue may generalize partially to a minor component of a compound discriminative stimulus. This is supported by the finding that the maximum 5-HT release occurs at 30 min and maximum DA release occurs at about 90 min (Yamamoto & Spanos, 1988). Interestingly, this change in peak neurotransmitter release corresponds to the WP reported in human subjects.

As the reader has surely noted by now, much of the drug discrimination research involving MDMA has conspicuously physiological overtones. Thus, a brief treatment touching on these effects should provide the reader with a great deal of helpful background that may be of considerable interest to the present study.

The Physiological and Neurochemical Effects of MDMA

MDMA has been shown to facilitate the presynaptic release of 5-HT (Johnson, Hoffman, & Nichols, 1986; Schmidt, Levin & Lovenberg, 1987; McKenna, Guan, & Shulgin, 1991) in rat brain synaptosomes (Nichols, Lloyd, Hoffman, Nichols, & Yim, 1982), caudate slices (Schmidt, Levin, & Lovenberg, 1987) and hippocampal slices (Johnson, Hoffman, & Nichols, 1986). DA release is also noted in the rat caudate nucleus (Johnson, Hoffman, and Nichols, 1986; Yamamoto & Spanos, 1988). Interestingly, MDMA has also been shown to inhibit the reuptake of 5-HT (Gold & Koob, 1989) and, to a lesser extent DA (Steele, Nichols, & Yim, 1987) into hippocampal synaptosomes.

High doses of MDMA have also been shown to decrease the firing rates of a subpopulation of 5-HT neurons in the dorsal and median raphe, leaving DA neurons unaffected. This implies that the effects of MDMA are mediated via a subpopulation of 5-HT neurons (Piercy, Lum, & Palmer, 1990). Interestingly, MDMA appears to facilitate DA release by way of 5-HT₂ receptor activity (Schmidt, Fayadel, Sullivan & Taylor, 1992).

The optical isomers of MDMA appear to have differential neurochemical effects. As mentioned earlier, the positive enantiomer of MDMA appears to be a more potent DA releaser than (-)-MDMA (Hiramatsu & Cho, 1990; McKenna et al., 1991; Johnson, Frescas, Oberlender, & Nichols, 1991) and (-)-MDMA binds to 5-HT receptors with higher affinity than (+)-MDMA (Lyon, Glennon, & Titeler, 1986).

Further, (+)-MDMA is not only more active than (-)-MDMA, but it is more active than the racemate as well (Anderson, Bronson, Braun, Nichols, & Shulgin, 1978). Interestingly, in a case study by Moore, Mozayani, Fierro, and Poklis (1996) helped determine that (+)-MDMA may metabolize more quickly than the negative isomer. When a 20 year old victim of fatal MDMA toxicity was subjected to autopsy, much more (-)-MDMA was found in the subject's system than was the positive enantiomer, indicating that--by the time of the victim's death--most of the (+)-MDMA had already been metabolized.

Mild MDMA toxicity seems to be characterized by agitation, tachycardia, hypertension, dilated pupils and sweating. Severe toxicity is often indicated by disseminated intravascular coagulation (DIC), hyperthermia, and acute renal failure (Henry Jeffreys, & Dawling, 1992). MDMA-related deaths are likely to be due to heat stroke, in which hyperthermia is accompanied by DIC (Henry, Jeffreys, & Dawling, 1992). Demirkiran, Jankovic, and Dean (1996) provided a summary of 16 MDMA-related case studies that indicates that MDMA toxicity occurs with a rather rapid onset (15 min to 6 h). More interestingly, their synopsis implied that toxicity, for some mysterious reason, does not seem to be well correlated with dose. One subject suffered severe toxicity within 15 min and died after taking a single tablet, where other subjects had managed to swallow some 47 pills (with an MDMA plasma level of 7.72 mg/l) with only hypertension and tachycardia as symptoms of toxicity. Still other

subjects have achieved a plasma level of 4.05 mg/l (18 tablets) with no complaints whatsoever, yet others have died with levels as low as 0.05-1.26 mg/l.

The Physiological Effects of MDMA Neurotoxicity

MDMA has also been demonstrated to have potentially neurotoxic effects (Stone, Johnson, Hanson, & Gibb, 1988) and the induction of this neurotoxicity may be accomplished by a plethora of administration methods. Short- and long-term decreases in 5-HT and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) concentrations, tryptophan hydroxylase (TPH, a rate-limiting step in 5-HT synthesis) activity, and degeneration of fine serotonergic axon terminals have been demonstrated in lab animals after subcutaneous (s.c.) (Commins, Vosmer, Virus, Woolverton, Schuster, & Seiden, 1987; Battaglia, Yeh, & DeSouza, 1988; Ricaurte, Forno, Wilson, DeLanney, Irwin, Molliver, & Langston, 1988) or oral (Ali, Newport, Bailey & Slikker, 1990; Ali, Newport, Scallet, Binienda, Ferguson, Bailey, Paule, & Slikker, 1993; Ali, Scallet, Newport, Lipe, Holson, & Slikker, 1989; Finnegan, Ricaurte, Ritchie, Irwin, Peroutka, & Langston, 1988; Ricaurte, DeLanney, Irwin, & Langston, 1988; Slikker, Ali, Scallet, Frith, Newport, & Bailey, 1988; Slikker, Holson, Ali, Kolta, Paule, Scallet, McMillan, Bailey, Hong, & Scalzo, 1989) administration of (±)-MDMA at relatively high doses (10-40 mg/kg for rats, 5-10 mg/kg for monkeys) for a few consecutive days (generally four). Ten to 20 mg/kg s.c. injections of (±)-MDMA elicits whole brain decreases of 5-HT, [³H]-5-HT uptake and TPH within 3

hours in rats--a decrement in 5-HT activity which is known to last for weeks even after a single dose (Schmidt, 1987, Schmidt & Kehne, 1990). Depletions of 5-HT (55%) and 5-HIAA (40%) in the frontal cortex, as well as in the whole brain (70%) of the rat are common findings in MDMA neurotoxicity studies (e.g., Slikker, Ali, Scallet, Frith, Newport, & Bailey, 1988). Robinson, Castaneda, and Whishaw (1993) noted a 72% decrease in whole-brain 5-HT levels (32.3% in the caudate nucleus) with no effect on dopamine (DA) levels in rats that were given i.p. injections of (\pm)-MDMA (10 mg/kg) every 12 hours for eight injections. Though this depletion was noted 35 to 40 days after the injection regimen completed, such long-term depletion by high, repeated doses of (\pm)-MDMA are not uncommon. Ricaurte and colleagues (e.g., Ricaurte, DeLanney, Irwin, & Langston, 1988; Ricaurte, Martello, Katz, & Martello, 1992) noted such effects up to 18 months after injection.

Apparently, multiple injections enhance this neurotoxic effect. Indeed, it has been noted that a multiple injection regimen results in the loss of immunohistologically labeled 5-HT terminals (but not cell bodies). The terminal loss achieved by this and many other neurotoxic regimens seems specific to the fine axons radiating from the dorsal raphe, sparing the beaded axons (Battaglia, Yeh, & DeSouza, 1988; Wilson, Ricaurte, & Molliver, 1989). Although systemic injection seems to be the most popular method of inducing neurotoxicity, other avenues have been explored. Oral MDMA (5-10 mg/kg, bid x 4 days) administration has been shown to decrease 5-HT and 5-HIAA levels in the hippocampus, hypothalamus, thalamus, and frontal cortex in

squirrel monkeys (Ricaurte, DeLanney, Irwin & Langston, 1988; Insel, Battaglia, Johanssen, Massa, & DeSouza, 1989). Ricaurte, DeLanney, Irwin, and Langston (1988) noted a decrease in 5-HT in the thalamus and hypothalamus two weeks after a single oral dose of 5 mg/kg MDMA. Similar findings have been noted in the hippocampus in rhesus monkeys after an oral dose of 2.5 mg/kg (Ali, Newport, Scallet, Binienda, Ferguson, Bailey, Paule, & Slikker, 1993). These doses appear to be remarkably low as compared to those previously discussed. Indeed, oral administration appears to be about half as effective in inducing neurotoxicity as s.c. administration in squirrel monkeys (Ricaurte, et al., 1988). However, nonhuman primates are known to be more sensitive to MDMA neurotoxicity than laboratory rodents (Steele, McCann, & Ricaurte, 1992; Ricaurte & McCann, 1992). Another note of interest is that the offspring of pregnant rats that were gavaged up to 10 mg/kg MDMA every other day from gestational days 6 to 18 did not demonstrate any neurotoxic effects (St. Omer, Ali, Holson, Durhart, Scalzo, & Slikker, 1991).

Any changes found after such administrations are often reported as evidence of neurotoxicity (Frederick, Ali, Slikker, Gillam, Allen & Paule, 1995). McKenna and Peroutka (1990) differentiated between what they called "long term" and "short term" neurotoxicity. Short term neurotoxicity occurs in less than 24 hours and is characterized by rapid decreases in 5-HT and its major metabolite 5-HIAA, as well as a corresponding decrease in TPH. Within two to four hours, however, 5-HIAA levels begin to return to normal. Long-term neurotoxicity, according to McKenna and

Peroutka, takes longer than 36 hours to take hold and is also characterized by a decrease in 5-HT, 5-HIAA, and TPH--only considerably slower. In this case, there is no recovery from long-term neurotoxicity as yet noted, and the aforementioned degeneration in 5-HT terminals is additionally noted. As noted earlier, this loss in 5-HT terminals is pretty well restricted to those on axons from cells originating in the dorsal raphe nucleus (O'Hearn, Battaglia, & DeSouza, 1988; Wilson, Ricaurte, & Molliver, 1989).

Interestingly, the effects of MDMA on DA levels are variable and believed to depend, indirectly, on serotonin release (Schmidt, Wu, & Lovenberg, 1986; Stone, Stahl, Hanson, & Gibb, 1986; Commins, Vosmer, & Virus, 1987; Yamamoto & Spanos, 1989; Gazzara, Takeda, Cho, & Howard, 1989), although DA seems to play a major role in this neurotoxicity (Glennon, Young, Rosencrans, Anderson, 1982). Increased levels of DA due to chronic administration of MDMA to monkeys have been found in the caudate nucleus, and a trend toward the increase of DA has been found in the frontal cortex and hippocampus of the monkey (Frederick, et al., 1995). Frederick et al. have also noted increased levels of 5-HT in the hippocampus, though 5-HT turnover was significantly decreased. This may be due to a decrease in hippocampal 5-HT receptor uptake sites or the lack of metabolism of 5-HT by monoamine oxidase (MAO) as compared to control monkeys. A decrease of 5-HT concentration was found, but turnover rate was unaffected in the frontal cortex of the subjects. Neither the frontal cortex, the caudate nucleus, nor the hippocampus showed

a decrease of 5-HT uptake sites as compared to those regions in control monkeys (Frederick et al., 1995). Also, MDMA neurotoxicity in squirrel monkeys has been noted to leave the major metabolite of DA, homovanillic acid (HVA) , and 3-methoxy-4-hydroxyphenethylglycol (MHPG), a major norepinephrine (NE) metabolite relatively unaltered (Ricaurte, DeLanney, Irwin & Langston, 1988; Insel, Battaglia, Johanssen, Massa, & DeSouza, 1989). Research with nonhuman primates, however, must be interpreted with a certain degree of care because, as noted earlier, they are more permanently and severely affected by MDMA neurotoxicity than rats and mice (Steele, McCann, & Ricaurte, 1992; Ricaurte & McCann, 1992).

Regardless of the species, however, it appears that MDMA neurotoxicity involves the 5-HT terminal regions of the hippocampus, neostriatum, and cerebral cortex in rodents as well as nonhuman primates (Ricaurte, Forno, Wilson, DeLanney, Irwin, Molliver, & Langston, 1988; Stone, Stahl, Hanson, & Gibb, 1987; Battaglia, Yeh, & DeSouza, 1988) with one interesting exception. A subtle inconsistency is noted between certain species when administered a neurotoxic MDMA regimen. Where a decrease in 5-HT and 5-HT terminal cell loss is evident in the rat while the DA system is somewhat unharmed, this is not the case with the mouse. In the latter organism, it is the DA system that is damaged, leaving the 5-HT system relatively intact (Stone, Hanson, & Gibb, 1987; Logan, Lavery, Sanderson, & Yee, 1988; O'Callaghan & Miller, 1994).

The Potential Causes of MDMA Neurotoxicity

The midbrain raphe nuclei provide the 5-HT innervation for the entire forebrain and have rather high concentrations of 5-HT receptor sites (Hrdina, Foy, Hepner, & Summers, 1990). Direct application of MDMA to this area *in vitro* increases 5-HT release (Sprouse, Bradberry, Roth, & Aghajanian, 1989) which eventually results in a decrease in cellular activity. Paris and Cunningham (1991) astutely noted that this may indicate that the dorsal raphe may be a target for MDMA neurotoxicity. However, intracerebroventricular (i.c.v.), injections of MDMA into the dorsal or median raphe did not yield any indications of neurotoxicity (Schmidt & Taylor, 1988; Paris & Cunningham, 1991).

In the light of such findings, it has been suggested that a metabolite of MDMA may be the prime mover in the brain to bring about neurotoxicity. However, its major metabolite, MDA, demonstrated as little neurotoxicity as MDMA when intracranially injected (Schmidt & Taylor, 1988). However, 2, 4-bis-(glutathion-s-yl)- α -methyldopamine, a putative metabolite of MDA--thus an indirect metabolite of MDMA--results in a neurotoxic syndrome when injected i.c.v. that is very similar to that seen with systemic MDMA administration (Miller, Lau, & Monks, 1997).

As mentioned previously it is likely that MDMA neurotoxicity may be partially due to DA action modulated by 5-HT activity. This contention is supported by the finding that although the 5-HT₂ agonist, 1-(2, 5-dimethoxy-5-iodophenyl)-2-aminopropane (DOI) increased sensitivity to neurotoxic MDMA dosing (Gudelsky,

Yamamoto, & Nash, 1994) while 5-HT depletion by p-chloramphetamine (PCA) did not protect against 5-HT axon terminal damage (Brodkin, Malyala, & Nash, 1993). However, DA depletion by α -methyl-p-tyrosine (AMPT) and lesions produced in the substantia nigra by 6-OHDA attenuate the long-term depletion of 5-HT in the brain (Brodkin, Malyala & Nash, 1993; Schmidt, Black, & Taylor, 1990; Stone, Johnson, Hanson, & Gibb, 1988). Thus, it is tempting to conclude that the neurotoxicity may be due to an excessive and prolonged exposure to DA elicited by the 5-HT release brought about by MDMA (Stone, et al., 1988; Nash, 1990). However, the search for the cause of MDMA neurotoxicity does not neatly end with such a convenient conclusion.

Hiramatsu, Kumagi, Unger, and Cho (1990) suggested that neurotoxicity might involve the production of oxygen-based free radicals during the breakdown of MDMA. During such a process, both O_2 and H_2O are produced (Graham, Tiffany, Bell, & Gutnecht, 1978; Cohen & Heikkila, 1974), which may produce an overabundance of oxidative stress that can contribute to the destruction of the 5-HT terminals. Of course, this may also occur during the breakdown of the excessive DA released by MDMA (Cadet, Landheim, Hirata, Rothman, Ali, Carlson, Epstein, & Moran, 1995).

MDMA Neurotoxicity In Humans

It is important to note at this point that physiologically functional impairments associated with MDMA neurotoxicity, it seems, are few and far between. Poland (1990) noted that adrenocorticotropin hormone release is decreased and prolactin is increased in rats after a neurotoxic treatment regimen of MDMA. Also, after neurotoxic dosing, rats appear to have an increased metabolic rate, evaporative water loss, and heightened colonic temperature (Gordon, Watkinson, O'Callaghan, & Miller, 1991). Such indications of MDMA-induced dehydration seems consistent with the toxicity found in humans at generally lower doses. Further, Gordon et al.'s (1991) observation that most of the rats (66%) died after a single dose of 20 mg/kg when their environmental temperatures were elevated (25-30°C) seem to mimic the unfortunate demise of the few human users in 1992 who have died in similarly warm environments (e.g., rave parties).

It is important to note, however, that it is presently uncertain as to whether MDMA is neurotoxic to humans. The reported serotonergic depletion induced by MDMA in primates and rodents is generally due to doses of the drug that are greater than those used by humans (Greer & Strassman, 1985). However, since the nonhuman primate is considerably more "at risk" for neurotoxicity than the laboratory rodent, and the dose for nonhuman primate neurotoxicity is only roughly double to the doses generally self-administered by humans (1.7 to 2.7 mg/kg in humans), some alarm has been raised as to the possibility of MDMA neurotoxicity in humans (Ricaurte,

DeLanney, Wiener, Irwin, & Langston, 1988). Indeed, there has been a considerable recent increase in reported psychiatric complaints by MDMA users such as anxiety, paranoid psychosis, and depression (Series, Boeles, Dorkins, & Peveler, 1994; McGuire & Fahy, 1991; McCann & Ricaurte, 1991).

However, determining whether or not a human has actually been exposed to a *neurotoxic* dose is difficult at best without performing postmortem examinations of the subject's brain tissue. Rather than passively waiting around for a given MDMA user to expire, a few researchers have attempted to verify MDMA neurotoxicity via CSF samples obtained by way of lumbar punctures in carefully screened MDMA users who have not taken the drug for an extended period of time (generally about two to six weeks). In this way, 5-HIAA or HVA levels may be measured, and neurotoxicity is assumed if long-term levels of these metabolites are observed. For example, Ricaurte, Finnegan, Irwin, and Langston (1990) found a decrease in 5-HIAA levels among MDMA users, but Peroutka, Pascoe, and Faull (1987) did not. McCann, Ridenour, Shaham, and Ricaurte (1994), however, detected potentially neurotoxic effects and some rather interesting gender effects as well. While all of the subjects had decreased levels of HVA and 5-HIAA relative to controls, female MDMA users had significantly lower levels of both metabolites than did their male counterparts.

Other studies have attempted to determine neurotoxicity in humans by way of the symptoms presumed to be manifested by a deficiency of 5-HT. One study (Allen, McCann, & Ricaurte, 1993) determined that there were significant differences in the

quality of sleep obtained by MDMA users after two weeks of abstinence than by non-users. MDMA users acquired 19 min less total sleep than did non-using control subjects. Users also spent 23.2 min less time in non-REM sleep and 37 min less in stage two sleep. However, it is important to interpret these findings with the thought in mind that no physiological measures of neurotoxicity were taken, and that neurotoxicity in these subjects was thus not confirmed.

Protection Against MDMA Neurotoxicity

An area of particular interest to the present study is the finding that MDMA neurotoxicity can be protected against. The compounds 6-nitroquipazine, paroxetine, and the benzylpiperazines p-nitrobenzylpiperazine, p-chlorobenzylpiperazine, and l-piperonylpiperazine, are all potent serotonin uptake inhibitors and have been shown to attenuate the 5-HT depletion associated with MDMA neurotoxicity (Hashimoto, Maeda, & Goromaru, 1992a, b). Further, the 5-HT uptake inhibitors MDL 11,939, citalopram and fluoxetine are some of the most frequently studied drugs that protect against the neurotoxicity of MDMA (Schmidt, Black & Taylor, 1990; Sprouse, Bradberry, Roth, & Aghajanian, 1989; O'Hearn, Battaglia, DeSouza, Kuhar, & Molliver, 1988; Azimitia, Murphy, & Whitaker-Azimitia, 1990; Schmidt, 1987). Interestingly, fluoxetine may provide this protection without affecting the discriminable effects of MDMA (McCann & Ricaurte, 1993). Protection by the noncompetitive N-methyl-D-aspartate calcium channel antagonist, MK-801, suggests

that the toxicity of MDMA may depend, at least in part, on disturbances in the homeostasis of the calcium channels of the 5-HT receptor population (Colado, Murray, & Green, 1993; Azimitia, Murphy, & Whitaker-Azimitia, 1990; Farfel, Vosmer, Seiden, 1992).

Serotonergically active drugs are not the only compounds that protect against MDMA neurotoxicity. The effects of MDMA neurotoxicity can be attenuated by the interruption of monoamine synthesis by monofluoromethyl DOPA, the depletion of vesicular monoamines via reserpine, or the inhibition of DA synthesis by AMPT or by 6-OHDA lesions in the substantia nigra (Schmidt, Black, & Taylor, 1990). This, plus the findings that the administration of the DA antagonist haloperidol or the monoamine oxidase inhibitor l-deprenyl also protects against MDMA neurotoxicity not only demonstrates that DA appears to be involved in MDMA neurotoxicity, but that the manipulation of the DA system may protect against it (Schmidt, Black & Taylor, 1990; Sprague & Nichols, 1995a, b).

The Behavioral Effects of MDMA Neurotoxicity

A great deal of research has obviously been performed on the physiological and neurotoxic effects of MDMA. Although some very salient behavioral effects of MDMA exist, there is a comparative paucity of research on the behavioral consequences of MDMA neurotoxicity. A few studies, however, have brought some of the most notable behavioral effects of MDMA neurotoxicity to light.

Although there is certainly no deficit in the reported physiological changes involved in MDMA neurotoxicity, corollary changes in behavior appear to be few and far between. Slikker, Holson, Ali, Kolta, Paule, Scallet, McMillan, Bailey, Hong, and Scalzo (1989) noted no significant changes in maze-learning or reflexive behavior. Ali, Newport, Scallet, Binienda, Ferguson, Bailey, Paule & Slikker (1993) noted no observable behavior change in rhesus monkeys observed in their home cage after neurotoxic dosing. Robinson, Castaneda, and Whishaw (1993) noted that rats after neurotoxicity had a longer acquisition latency in negotiating a water maze, but no changes in foraging or "skilled reaching". In the skilled reaching test, food acquisition was contingent on the rats' ability to reach the food bin, which was placed a considerable distance on the other end of an aperture. However, although no neurotoxicity was found in pups that were prenatally exposed to a neurotoxic MDMA regimen, St. Omer et al. (1991) noted that these young rats developed better olfactory discrimination and that negative geotaxis (180° rotation within 60 s after being placed facing down a 25° incline) was delayed in the female offspring.

Also of particular importance to the present paper, it has been established that neurotoxicity attenuates the discriminative stimulus control exerted by MDMA. Schechter (1991b) found that when para-chlorophenylalanine (p-CPA), a competitive TPH inhibitor was injected daily for three days (100 mg/kg, i.p.) in rats trained to discriminate (±)-MDMA from saline, the discrimination of the training compound was disrupted. Similarly, a neurotoxic dose of fenfluramine (4.0 mg/kg, i.p., bid x 4 days)

was found to attenuate the discriminative stimulus control of (±)-MDMA in one study (Baker & Makhay, 1996). Curiously, amphetamine, a drug that had heretofore only substituted for MDMA on irregular occasions, substituted rather well for (±)-MDMA after the rats had endured a neurotoxic dose regimen in the Baker and Makhay (1996) study. Finally, Schechter (1991a) demonstrated that although a neurotoxic regimen of (±)-MDMA did not affect the conditioned place preference engendered by (±)-MDMA, discrimination of the drug had been strongly decremented.

The Purpose of the Present Study

Since the optical isomers of MDMA appear to be drastically distinct from each other and the racemate, it was of particular interest to delineate the effects of (±)-MDMA-induced neurotoxicity on the discriminative stimulus properties of each isomer individually. Further, although it is well established that the 5-HT reuptake inhibitor, fluoxetine, protects against the neurochemical aspects of MDMA neurotoxicity, its effects on the behavioral aspects of neurotoxicity, specifically the discriminative stimulus control exerted by the isomers of MDMA, have yet to be established. Thus, the present study was designed to provide such information by the administration of a neurotoxic (±)-MDMA regimen, with or without concomitant fluoxetine injections, to rats trained to discriminate either (+) or (-)-MDMA from saline in a two-lever drug discrimination procedure.

CHAPTER II

METHODS

Subjects

Thirty male Sprague-Dawley rats (Harlan Breeding Laboratories, Harlan, IN) weighing 390-450 g at the beginning of the study were used. Animals were housed individually in a temperature- and humidity-controlled colony (22-29°C and 39-50%, respectively) maintained on a 12 h light-dark schedule (0700 h-1900 h light). Standard laboratory rodent diet was available ad libitum. Access to water was restricted to amounts obtained during training sessions, for 10-15 minutes following training and test sessions, and for at least 24 h on weekends. Twenty-four rats were randomly divided into two groups of 12 and each group was assigned to be trained to discriminate (-)-MDMA or (+)-MDMA. Each group of 12 rats was further divided into groups of six. Rats in each group in each isomeric condition received concomitant injections either saline and (±)-MDMA (MDMA) or fluoxetine and (±)-MDMA (FLX + MDMA) during the neurotoxic drug administration regimen. Thus, four groups of six rats were formed, consisting of rats trained to discriminate (-)-MDMA or (+)-MDMA that were exposed to MDMA or FLX + MDMA conditions.

The remaining six subjects were maintained as drug and experimentally naive controls for postmortem neurochemical analysis. Two of the 12 rats exposed to (-)-MDMA died unexpectedly during discrimination training, and seven died during the neurotoxic drug administration regimen, leaving three rats to complete the study in the (-)-MDMA condition.

Apparatus

Training and testing were conducted in eight commercially available chambers (Med-Associates standard operant chamber ENV-001), housed in sound- and light-attenuating shells, which provided ventilation and masking noise. Each chamber contained a 28 V house light and dipper (0.1 ml) mounted equidistant between two levers. A Zenith Z-320/SX microcomputer, located in an adjacent room, controlled experimental events and data collection via interface and software (Med-Associates, East Fairfield, VT).

Drugs

The drugs used in the present experiment were dissolved in 0.9% bacteriostatic saline and administered s.c. in a volume of 1 ml/kg. The drugs and dose ranges were: (+)-MDMA hydrochloride (0.375, 0.75, and 1.5 mg/kg), (-)-MDMA hydrochloride (0.75, 1.0, 1.5, 2.0, 3.0, and 4.0 mg/kg), (±)-MDMA hydrochloride (10, 20 mg/kg) and fluoxetine hydrochloride (5.0 mg/kg). MDMA and its stereoisomers were

generously granted from the National Institute on Drug Abuse (NIDA, Providence, RI) and fluoxetine was the charitable gift of Eli Lilly (Indianapolis, IN).

Behavioral Procedures

Training

Subjects were initially trained to discriminate (+)-MDMA (1.5 mg/kg, n = 12) or (-)-MDMA (3.0 mg/kg, n = 12) from saline in a two lever, water reinforced drug discrimination task. Injections of drug or saline were administered 20 min prior to 20 min sessions which were run six days per week. Drug or saline was given randomly, with the restriction that neither condition occur for more than two consecutive days. Half of the animals were reinforced with water for responses on the left lever following drug, and reinforced on the right lever following saline injections; conditions were reversed in the remaining animals. The number of consecutive correct responses required for reinforcement was gradually increased from 1 to 20 until all animals maintained reliable rates of responding under an FR 20 schedule. Reinforcement was conditional upon the completion of the FR on the correct lever (drug or saline); responding on the incorrect lever was recorded and resulted in the resetting of the FR 20 schedule, but had no additional programmed consequences. To reduce olfactory cues (c.f., Extance & Goudie, 1981), the levers were wiped with an isopropyl alcohol solution before each session. Further, the order in which the animals received training

was randomized. When animals attained a criterion of 80% correct prior to the delivery of the first reinforcer on 8 out of 10 consecutive sessions, testing began.

Dose Response Testing

Animals were administered substitution tests with several doses of the training drug (0, 0.375, 0.75, or 1.5 mg/kg (+)-MDMA; 0, 0.75, 1.5, or 3.0 mg/kg (-)-MDMA) which, were administered in place of the training drug. Test sessions terminated without reinforcement following the completion of the 20 consecutive responses on either lever or after 20 min had elapsed. Test sessions were conducted once or twice per week in animals that maintained a minimum of 80% for at least two consecutive training sessions (i.e., drug and saline) between tests. After all doses were tested, sham neurotoxic dosing began.

Sham Injection Regimen

All subjects were administered two injections of vehicle twice daily (10 a.m. and 10 p.m.) for four days. The rats were not trained or tested during this period, nor for 9 days afterward. On the fourteenth day following the initial injection, half of the rats were tested for stimulus generalization to the training dose and the other half were exposed to a similar test using vehicle. The following day, the rats were tested again with reversed conditions (i.e., the rats that were exposed to a saline substitution test the previous day were tested for generalization to the training dose, and vice versa).

The following day, the rats were exposed to training sessions until discrimination criterion was met again, at which point the (\pm)-MDMA neurotoxic administration began.

Rats trained to discriminate (-)-MDMA, however, failed to maintain discrimination after this regimen and were retrained per the above procedure to discriminate (-)-MDMA at a slightly higher dose (4.0 mg/kg). A dose response curve was then reestablished as above using slightly different doses (0, 1.0, 2.0, and 4.0 mg/kg) and a second sham neurotoxic injection regimen was implemented, followed by a second generalization test. Rats undergoing this second test were tested twice on each condition (training dose and vehicle) in an alternated order similar to that described above and the average of each condition was taken. After this testing procedure, the rats were exposed to testing sessions again until the discrimination criterion were met, at which point the neurotoxic regimen was implemented.

Neurotoxic Injection Regimen

Under this condition, rats were administered 20 mg/kg (\pm)-MDMA twice daily for four days (c.f. Schechter, 1991) on a 12 h schedule (10 a.m. and 10p.m.). Half of the rats in each isomeric condition were administered 5.0 mg/kg fluoxetine a few seconds before each (\pm)-MDMA injection. Similar to the sham neurotoxic administration procedure, the rats were not trained or tested during this time or for the

next 9 days. They were then exposed to alternated saline/training drug substitution tests and retrained until discrimination criterion was met once more.

Initially, eight of the ten surviving rats in the (-)-MDMA condition were administered the described neurotoxic dose. However, seven of these rats died by the third injection. The single surviving rat completed this dosing regimen at a lower dose of (±)-MDMA (10 mg/kg) and the remaining two were administered this lower dose at each injection of the neurotoxic procedure. Consistent with the sham neurotoxic procedure, these three rats were exposed to two alternated tests of saline/training drug substitution tests each and the mean performance calculated for both testing conditions. After this testing procedure, the rats were retrained until criterion was met again.

Postmortem Neurochemical Assays

After the third discrimination criterion was met, the rats were given a seven day drug holiday with food and water available *ad libitum*. On the eighth day after criterion was met, the rats were sacrificed by decapitation via a commercially available rat guillotine (Braintree Scientific, Braintree, MA). The brains were rapidly removed and rinsed with ice-cold saline and placed in a chilled cutting block. The ventral striatum as well as the prefrontal cortex were removed and placed in separate tubes and frozen on dry ice. Samples were stored at -80°C until they were analyzed for monoamine content at the facilities of Pharmacia & Upjohn (Kalamazoo, MI). The

sections were then homogenized via a sonifier in a 500 ml buffer of a 10% solution of EDTA, 5% glutathione, and 0.01 N PCA and centrifuged at 14,000 r.p.m for 14 min at 4° C. The supernate was then drawn off and assayed by HPLC and electrochemical detection.

Data Analysis

Percent drug lever responding was defined as the number of responses emitted on the lever associated with the training drug, divided by the total number of responses that occurred during a test session (X 100). Rate (responses per second) was the total number of responses that occurred during a test, divided by the number of seconds taken to complete the test. Data from animals that failed to complete 20 responses during stimulus generalization tests were discarded. The criteria for complete substitution were: (a) a mean of at least 80% drug-appropriate responding; and (b) that this mean was significantly different from that of saline control tests.

Sessions to criterion (STC) was defined as the number of training sessions required for a given subject to achieve criterion. STC was recorded at three points: during initial training prior to the dose response testing (STC 1), during retraining after the sham neurotoxic administration (STC 2), and during retraining after the neurotoxic administration regimen (STC 3).

The effects of each substitution dose during the dose response analysis on each of these measures were subjected to a one way repeated measures analysis of variance (ANOVA). The effects of training drug/saline administration during generalization tests

after sham or neurotoxic injections were analyzed via a 2-way ANOVA (Group x Time). The Group factor refers to the regimen after which testing was performed (sham injection regimen vs. neurotoxic injection regimen). Where appropriate, Tukey's multiple comparison test was used. Statistical analyses were performed using GraphPad Prism software (Prism, San Diego, CA). Due to differential loss of subjects in the (-)-MDMA condition, data analyses were not performed with data from that isomer, save for the dose response analyses for each training drug. Data collected from the postmortem neurochemical assays were subjected to one-way ANOVA analyses.

CHAPTER III

RESULTS

(+)-MDMA

Dose Generalization

Figure 2 illustrates the dose dependent nature with which (+)-MDMA evoked drug-appropriate lever responding in rats trained to discriminate 1.5 mg/kg (+)-MDMA ($F(3,33) = 18.53, p < 0.0001$). Most notable is that the training dose substituted completely for itself with a mean of 98.35% (SEM = 1.29) and saline injections engendered little, if any, drug appropriate responding in the same rats (mean = 0.725, SEM = 0.725). Doses of 0.375 and 0.75 mg/kg (+)-MDMA evoked %38.37 (SEM = 12.71) and 51.79% (SEM = 13.87) (+)-MDMA-appropriate responding, respectively. Tukey's multiple comparison test indicated that the percent (+)-MDMA lever responses engendered by saline was significantly lower than that of all other drug doses ($p < 0.05$) and, conversely, drug-appropriate responding was much greater when rats were injected with the training dose than with any other dose of (+)-MDMA ($p < 0.05$).

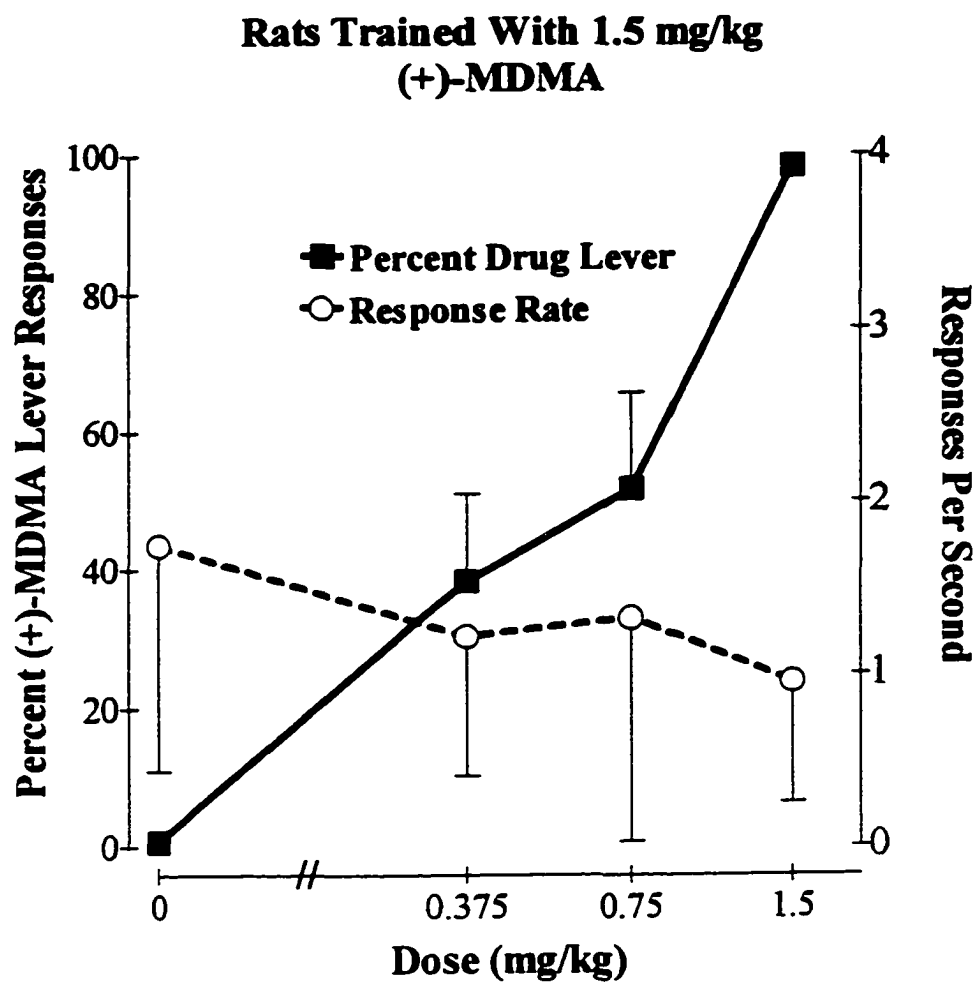


Figure 2. Percent (+)-MDMA Responses and Rate of Response During Substitution Tests in Rats Trained to Discriminate (+)-MDMA From Saline.

Response rate also appeared to be affected by drug dose ($F(3,33) = 2.93$, $p = 0.048$), which engendered mean rates of 1.74 (Saline, SEM = 0.219), 1.22 (0.375 mg/kg, SEM = 0.2), 1.32 (0.75 mg/kg, SEM = 0.27), and 0.95 (1.5 mg/kg, SEM = 0.22) responses per second. Tukey's multiple comparison test, however, indicated that no dose condition differed from any other in this measure.

Response Testing After Sham and Neurotoxic Injection Regimens

Figure 3 illustrates that, after the sham injection regimen, the training dose of (+)-MDMA substituted for itself in the rats in the MDMA group (99.24%, SEM = 0.76) and those in the FLX + MDMA group (100.0%, SEM = 0.0). Conversely, saline evoked very little, if any, (+)-MDMA responses from these rats in either condition (MDMA: 2.78%, SEM = 2.78; FLX + MDMA : 9.42%, SEM = 4.27). After the neurotoxic drug administration regimen, however, (+)-MDMA only promoted criterion level drug-appropriate responding in rats that were concomitantly administered fluoxetine with MDMA (100.0%, SEM = 0.0), as opposed to rats in the MDMA group (44.99%, SEM = 19.74). Similarly, saline engendered more drug-like responding in rats in the MDMA group (22.14%, SEM = 10.63) than in the FLX + MDMA group (2.31%, SEM = 1.56). A two way ANOVA of Group (MDMA vs. FLX + MDMA) and Time (after sham injection regimen vs. after neurotoxic injection regimen) was performed with the data from the training dose substitution tests. This test indicated a significant interaction between the Group and Time factors ($F(1,20) =$

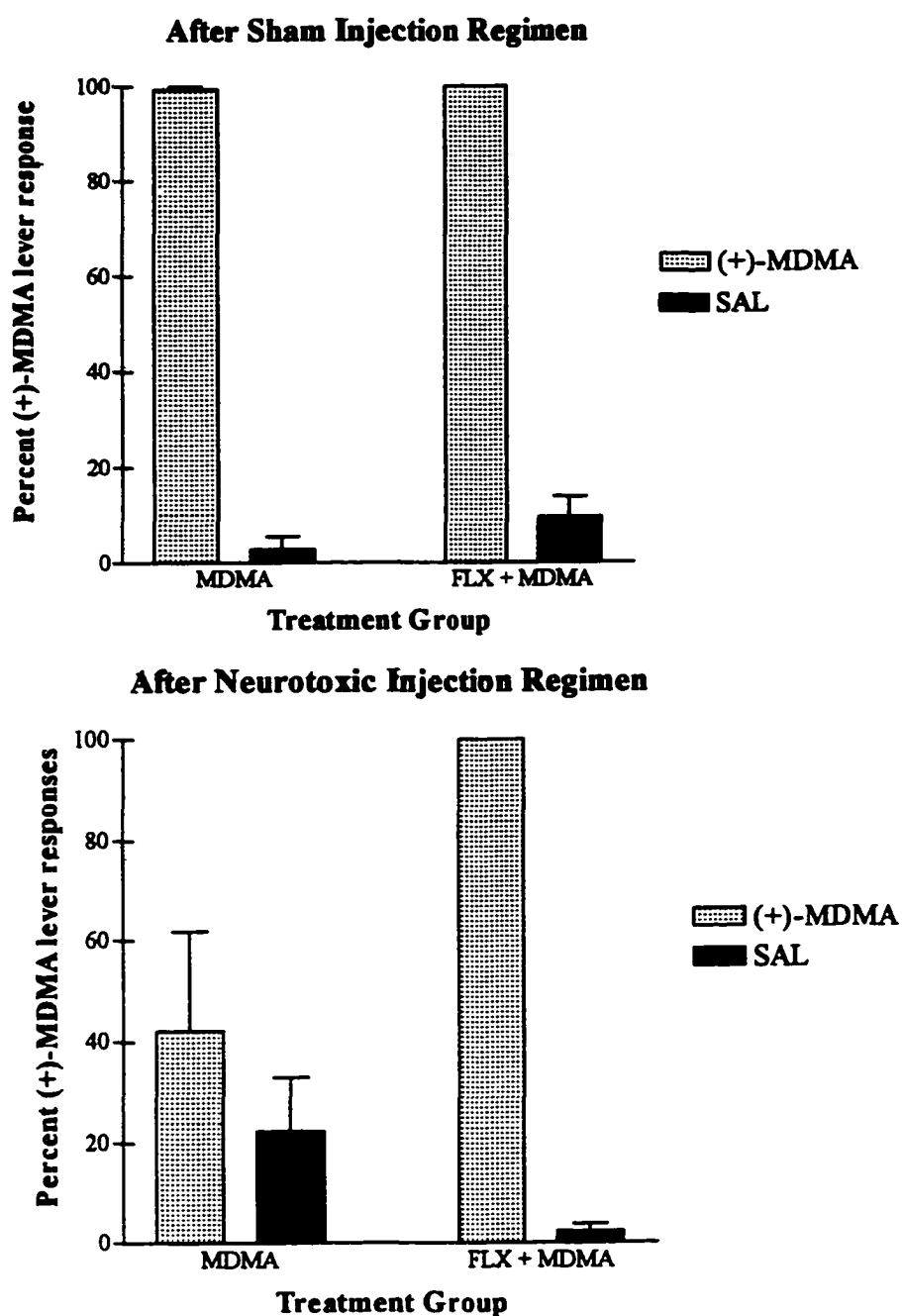


Figure 3. Percent (+)-MDMA Responses During Training-Dose Substitution Tests after Sham and Neurotoxic Injection Regimens.

8.4, $p = 0.0089$) and a significant main effect in both the Group ($F(1,20) = 8.85$, $p = 0.0075$) and the Time ($F(1,20) = 8.4$, $p = 0.0089$) factors. A similar analysis of the data from the saline substitution tests indicate only a significant interaction between the two factors ($F(1,20) = 4.95$, $p = 0.038$) and no significant main effect.

Figure 4 demonstrates the effects of (\pm)-MDMA neurotoxicity and fluoxetine on response rate. After the sham injection regimen, response rates engendered in the rats in the MDMA group during saline and (+)-MDMA training dose substitution tests were 1.77 (SEM = 0.45) and 0.89 (SEM = 0.196) responses per second, respectively. Among the FLX + MDMA rats, saline substitution tests engendered 0.83 (SEM = 0.31) responses per second and (+)-MDMA training dose substitution tests engendered 0.365 (SEM = 0.07) responses per second. After the neurotoxic injection regimen, response rates during (+)-MDMA substitution tests were 0.89 (SEM = 0.23) among rats in the MDMA condition and 0.997 (SEM = 0.2) in FLX + MDMA rats. Response rates among rats in the MDMA and FLX + MDMA conditions during training dose substitution tests were 1.25 (SEM = 0.282) and 0.895 (SEM = 0.122) responses per second, respectively. Two way ANOVA's of the data obtained from these tests indicated no significant interaction or main effect in this measure.

Sessions to Criterion (STC)

The number of sessions to criterion (STC) prior to dose response testing (STC 1) was 31.39 (SEM = 1.47) among rats in the FLX + MDMA group and 33.33 (SEM

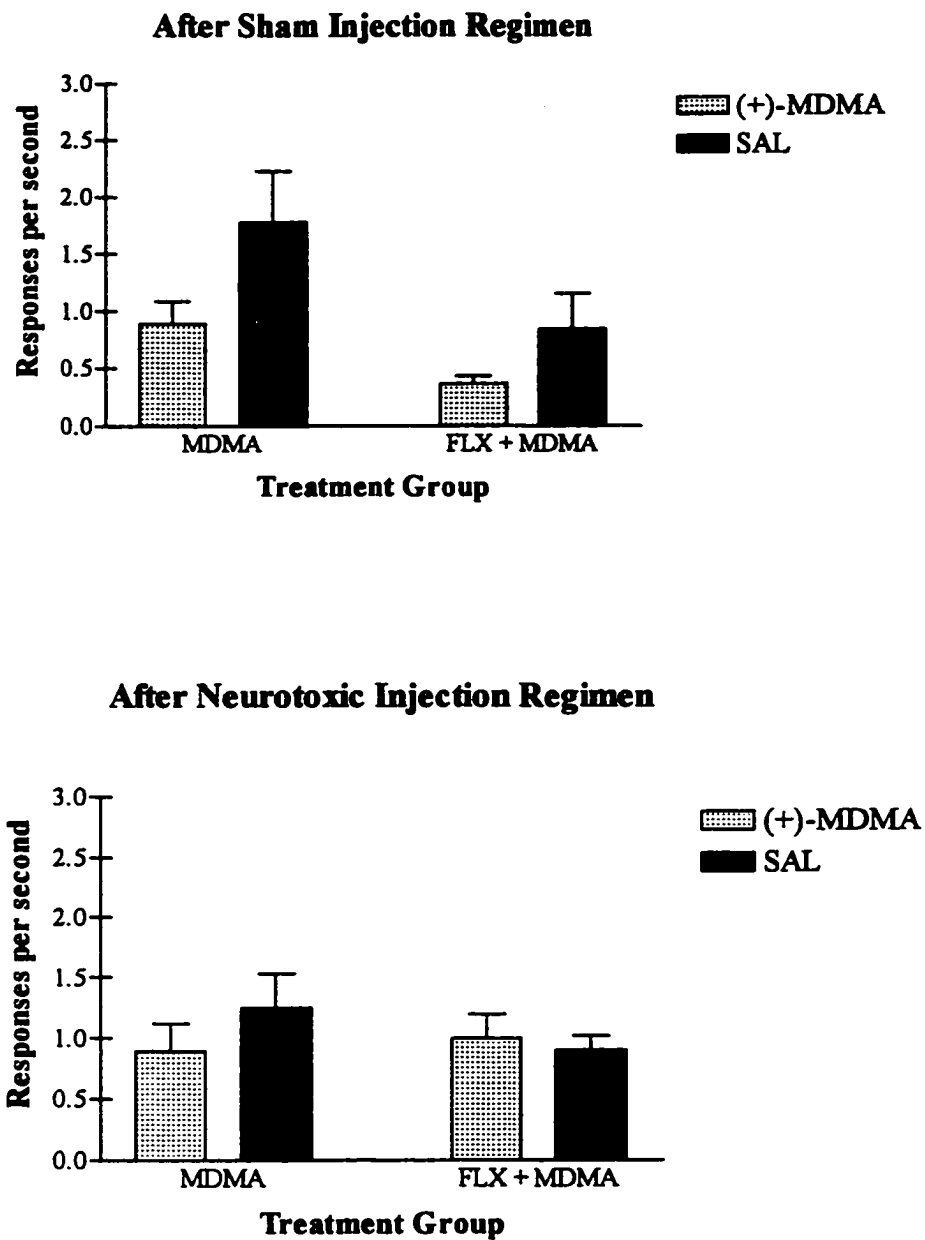


Figure 4. Response Rate During Training-Dose Substitution Tests After Sham and Neurotoxic Injection Regimens.

= 1.33) among the rats in the MDMA group (Figure 5). Figure 5 also illustrates the STC after chronic saline injections (STC 2) among MDMA (11.0, SEM = 1.0) and FLX + MDMA (11.17, SEM = 1.17) rats, respectively. The STC after the neurotoxic dosing regimen (STC 3) was 10.0 (SEM = 0) among the rats in the FLX + MDMA condition and 17.0 (SEM = 4.82) in the subjects in the MDMA group. A two-way ANOVA (Group vs. STC period) indicated no significant interaction and no main effect for the Group factor. There was, however, a significant main effect in the STC period factor ($F(2,30) = 63.93$, $p < 0.0001$). That is, STC's were lower at STC 2 and STC 3 than at STC 1.

Postmortem Neurochemical Assays

A significant main effect was found in the 5-HT levels in the prefrontal cortex areas of the subjects ($F(2, 14) = 9.04$, $p = 0.003$). Serotonin levels among rats in the MDMA group were significantly lower than those in the rats in the Control ($p < 0.01$) and the FLX + MDMA groups ($p < 0.05$). A similar main effect in rats in the 5-HIAA levels of the subjects' prefrontal cortices ($F(2, 24) = 11.88$, $p = 0.001$). 5-HIAA levels among the rats in both the MDMA ($p < 0.05$) and the FLX + MDMA ($p < 0.05$) groups were significantly lower than those found in the rats in the Control group. No significant differences were found among any of the other neurotransmitters detected, nor were any differences found among the striata of the rats in any condition (Figure 6).

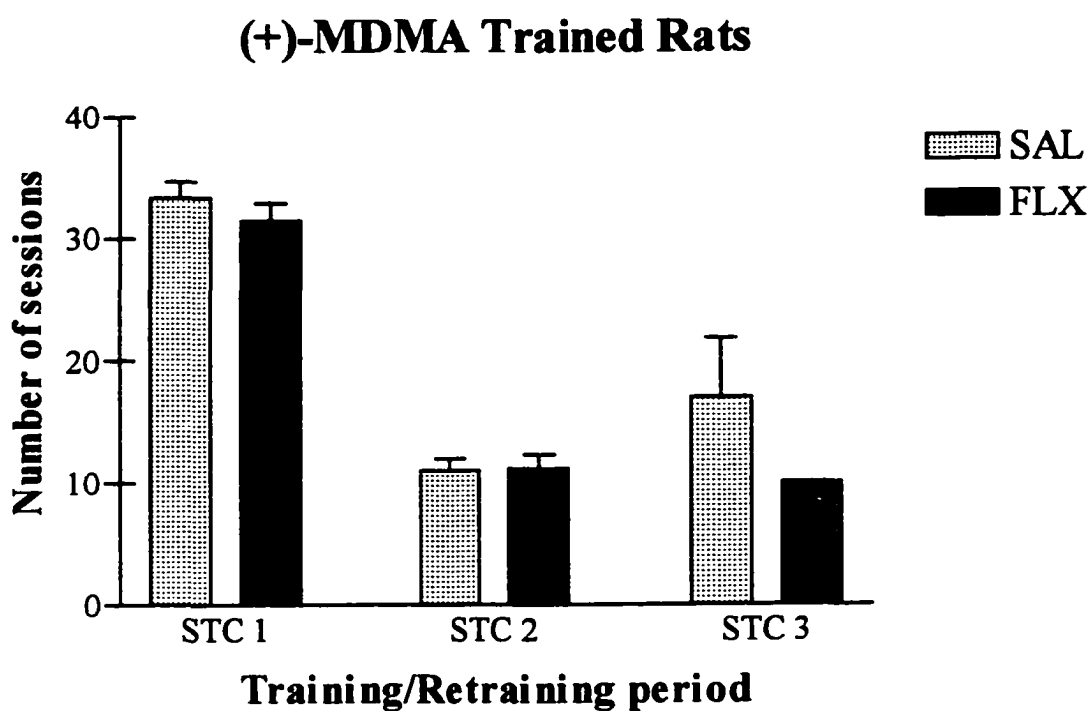


Figure 5. Number of Sessions Prior to Criterion Preceding Dose Response Testing (STC 1) After Sham Injection Regimen (STC 2) and After Neurotoxic (\pm)-MDMA Administration (STC 3).

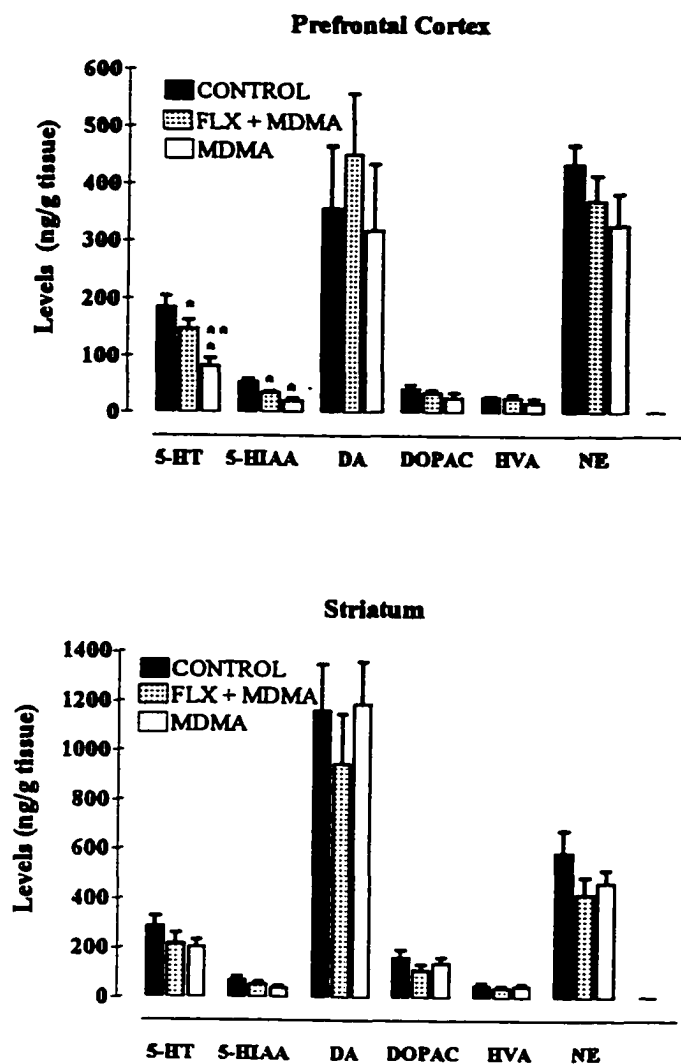


Figure 6. Neurotransmitter and Major Metabolite Levels Detected in the Prefrontal Cortices and Striata of Rats Under Control, FLX + MDMA, and MDMA Conditions.

(-)-MDMA

Dose Generalization With 3.0 mg/kg as a Training Dose

(-)-MDMA evoked drug-appropriate responding in a dose-dependent manner in rats trained to discriminate 3.0 mg/kg (-)-MDMA ($F(3, 36) = 6.97$, $p = 0.0008$, Figure 7). Of primary interest is that the training dose substituted for itself (81.82%, SEM = 12.197) and saline engendered little (-)-MDMA-like lever responding (6.96%, SEM = 3.76). Substitution tests of doses of 0.75 and 1.5 mg/kg (-)-MDMA resulted in 37.58% (SEM = 13.61) and 42.61% (SEM = 15.06) drug appropriate responses, respectively. Tukey's multiple comparison test indicated that the percent (-)-MDMA lever responses engendered by the training dose was significantly greater than that evoked by saline ($p < 0.001$).

Response rate, however, was unaffected by drug dose. The mean responses per second for substitution tests of saline, 0.75, 1.5 and 3.0 mg/kg (-)-MDMA were 2.2 (SEM = 0.81), 1.14 (SEM = 0.22), 1.32 (SEM = 0.17), and 1.74 (SEM = 0.33) responses per second, respectively (Figure 7).

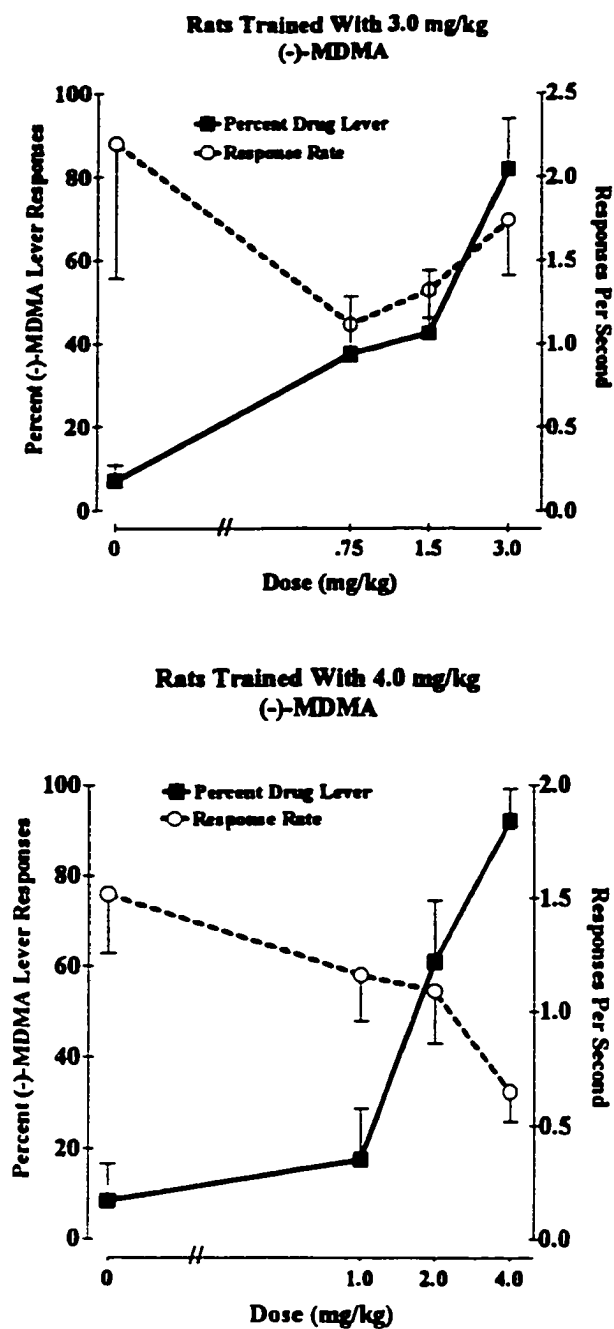


Figure 7. Percent (-)-MDMA Responses and Rate of Response During Substitution Tests in Rats Trained to Discriminate 3.0 mg/kg and 4.0 mg/kg (-)-MDMA From Saline.

Dose Generalization With 4.0 mg/kg as a Training Dose

Figure 7 also illustrates the dose dependent nature with which (-)-MDMA engendered drug appropriate responding in rats trained to discriminate 4.0 mg/kg (-)-MDMA from saline ($F(3,33) = 21.1$, $p < 0.0001$). Consistent with the above findings, the training dose substituted for itself (91.96%, SEM = 7.194) and saline substitution resulted in less than criterion (-)-MDMA lever responses (8.33%, SEM = 8.33). Substitution tests with the intermediate doses of 1.0 and 2.0 mg/kg (-)-MDMA yielded 17.5% (SEM = 11.14) and 60.94% (SEM = 13.48) (-)-MDMA appropriate responding, respectively. Tukey's multiple comparison analysis indicated that saline engendered significantly less drug appropriate responding than 2.0 and 4.0 mg/kg (-)-MDMA ($p < 0.001$). Further, 1.0 mg/kg administration resulted in significantly less drug appropriate responding than 2.0 mg/kg ($p < 0.01$) and 4.0 mg/kg ($p < 0.001$).

Response rate was reduced by increasing doses of (-)-MDMA ($F(3,33) = 5.75$, $p = 0.003$), which engendered rates of 1.52 (saline, SEM = 0.262), 1.16 (1.0 mg/kg, SEM = 0.202), 1.09 (2.0 mg/kg, SEM = 0.23), and 0.65 (4.0 mg/kg, SEM = 0.13) responses per second, respectively. Tukey's multiple comparisons indicates that the saline substitution test resulted in a significantly higher rate than did the training dose ($p < 0.01$).

CHAPTER IV

CONCLUSIONS

The present results are consistent with previous finding (e.g., Baker et al., 1997; Baker et al., 1995) that discriminative stimulus control can be established with both isomers of MDMA and that this control provides a dose-dependent generalization gradient. Further, the present study established the ability of (+)-MDMA to maintain its discriminative stimulus properties over an extended period in which the subjects were not exposed to training sessions. This training hiatus is inherent in the commonly used neurotoxic drug administration procedure utilized herein, and thus any conclusions based thereon can safely be disentangled from the procedural cessation in training (c.f., Schechter, 1991a). It is interesting to note that the present findings also established that such discriminative control may be established via subcutaneous injections at doses where it has, up to this point, primarily been achieved through a route of more immediacy and invasiveness (i.e., intraperitoneal injections).

The neurochemical analysis of the prefrontal cortices of rats undergoing (±)-MDMA neurotoxicity demonstrated a significant decrease in 5-HT and its major metabolite, 5-HIAA, with little effect on the dopaminergic and noradrenergic systems relative to control. This finding is consistent with previous research (e.g., Slikker et al, 1988; Robinson, et. al., 1993) and supports the contention that (±)-MDMA

neurotoxicity contributed to the disruption of the discriminative stimulus control of (+)-MDMA. It is well-established that serotonergic depletion via neurotoxic administration of 5-HT-mediated drugs (e.g., fenfluramine, *p*-chlorophenylalanine, MDMA) deteriorates the discriminative control achieved with (±)-MDMA (Baker & Makhay, 1996; Schechter, 1991a, b). The same has been found for other serotonergically mediated drugs, including MDE and fenfluramine (Schechter, 1991b). Thus, it appears that depletions within the serotonergic system significantly decrease the ability of rats to discriminate centrally active drugs whose mechanism of action primarily involves 5-HT activity. The present study extends this finding to include the (+) isomer of this compound and further establishes that, although the neurotoxicity may be permanent, discrimination of the training dose may be reestablished with little or no loss of discriminative stimulus control (c.f., Baker & Makhay, 1996).

Many factors may contribute to this phenomenon. It is possible that, since (+)-MDMA is dopaminergically as well as serotonergically active (McKenna, Guan, & Shulgin, 1991), that the discriminative control of this isomer may have been reestablished with its dopaminergic properties playing a salient role. This is somewhat supported by the findings of Baker and Makhay (1996), which noted that, after 5-HT depletion by fenfluramine, the dopaminergically active drug, amphetamine, substituted for (±)-MDMA. The substitution of amphetamine for MDMA in trained rats was, until then, tentative at best. Further, Schechter (1997) noted that Fawn-Hooded rats--an inbred strain of rats with an indogenous 5-HT storage deficit--showed no deficit in

MDMA discrimination relative to Sprague-Dawley rats. It can also be argued, however, that since the serotonergic depletion by MDMA neurotoxicity is incomplete, the subjects may indeed be discriminating the drug on the basis of its serotonergic properties, but at a functionally lower dose.

Of primary interest is that, though temporary with subsequent training, this loss in the discriminative control by (+)-MDMA can be prevented. The postmortem neurochemical analysis in the present study indicates that 5-HT levels were left unaltered in the prefrontal cortices of rats given fluoxetine injections concomitant with (±)-MDMA injections that would, normally, result in neurotoxicity in rats. By providing concurrent administrations of fluoxetine, a drug commonly known to protect against MDMA neurotoxicity (e.g., Schmidt, Black & Taylor, 1990), the discriminative stimulus control exerted by (+)-MDMA can be preserved. This finding provides heretofore unestablished information regarding the behavioral effects of MDMA neurotoxicity--that it is possible that the behavioral deficits due to MDMA neurotoxicity may be hindered along with the corollary biochemical events associated with such neurotoxic treatment. The present finding is limited, however, by the relative paucity of research regarding the behavioral effects of MDMA neurotoxicity and even more so by the lack of behaviors that have been found to be thus affected.

It is unfortunate that the subjects that were trained to discriminate 3.0 mg/kg (-)-MDMA from saline failed to maintain discrimination after the training recess in the sham neurotoxic procedure. Since the discrimination of similar doses of this isomer

through i.p. injections is well established, (e.g., Baker, et al., 1997) it is likely that, due to the subcutaneous nature of the current drug administration route, the dose was not high enough in this less potent isomer to maintain discriminative stimulus control after an extended break in training. However, since no attempts have been previously made to determine whether discriminative stimulus control by (-)-MDMA at that dose administered by any route can be maintained over a period of no training, and that s.c. injections of 3.0 mg/kg (-)-MDMA substituted perfectly in rats trained to discriminate i.p. injections of the same dose of the drug from saline (Baker & Virden, unpublished results), it is obvious that more research is necessary prior to making any conclusions in that regard.

More's the pity that, after the requisite discrimination maintenance of a higher dose of (-)-MDMA was established, the neurotoxic regimen of (\pm)-MDMA proved toxic to most of the exposed rats, causing a significant differential depletion of subjects in that condition, and rendering any analysis of the results obtained from them futile. This near-tragic loss may be attributable to a number of causes. It is possible that since the rats were exposed to considerably more injections of the training drug (by 47-50 injections) than their (+)-MDMA counterparts, due to retraining and the reestablishing of a dose response curve at a higher training dose, that these rats may have become sensitized to the potentially toxic effects of the higher doses of the racemate. Further, it is also conceivable that, since the rats in the (-)-MDMA group were somewhat older than the (+)-MDMA subjects (by 110-117 days), by the time the

received the neurotoxic regimen, that the rats' relative age may have played an important role in their deaths. It is more likely, however, that the unexpected deaths of these subjects were the result of an unforeseen aberration in environmental factors. It is known that high temperatures (25-30°C) can facilitate the lethality of the dose of (±)-MDMA employed in the present procedure (Gordon, Watkinson, O'Callaghan, & Miller, 1991). Indeed, the range of room temperatures during the days of injection for this group (25-29°C) was similar to that found to be fatally interactive with (±)-MDMA by Gordon et al., (1991), and was considerably higher than the temperatures during neurotoxic injection of the rats in the (+)-MDMA group (22-24°C), all of which survived with no unexpected complications. However, these factors are difficult to disentangle and, again, more research is necessary before any conclusions can be safely made.

It can be argued that this relative paucity in research may be due to a lack of sensitive means by which to detect functional MDMA neurotoxicity. Schechter (1991a) demonstrated that the drug discrimination procedure may be an avenue through which it may be quantified. Further, the present study has demonstrated that this procedure may detect the preservation of behavioral functions against MDMA neurotoxicity, thus facilitating further research in this field. In summary, the present findings indicate that the discriminative stimulus control of (+)-MDMA as disrupted by (±)-MDMA neurotoxicity can be established, regained, and protected against. Although there is a present lack of research involving the behavioral effects of

MDMA neurotoxicity, the present study underscores the need for such explorations, and brings to light a potentially important tool with which it may be accomplished.

Appendix

Protocol Clearance From the Institutional Animal Care and Use Committee (IACUC)

**WESTERN MICHIGAN UNIVERSITY
INVESTIGATOR IACUC CERTIFICATE**

Title of Project: Discriminative Stimulus Effects of MDMA: Effects of Fluoxetine on Neurotoxicity

The information included in this IACUC application is accurate to the best of my knowledge. All personnel listed recognize their responsibility in complying with university policies governing the care and use of animals.

I declare that all experiments involving live animals will be performed under my supervision or that of another qualified scientist. Technicians or students involved have been trained in proper procedures in animal handling, administration of anesthetics, analgesics, and euthanasia to be used in this project.

If this project is funded by an extramural source, I certify that this application accurately reflects all procedures involving laboratory animal subjects described in the proposal to the funding agency noted above.

Any proposed revisions to or variations from the animal care and use data will be promptly forwarded to the IACUC for approval.

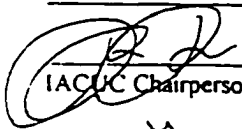
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_____ Approved

☒ Approved with the provisions listed below

Provisions or Explanations:

Provide a specific Database (e.g. Medline) which
was searched to insure no duplication.



IACUC Chairperson

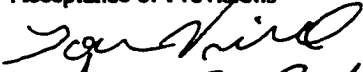
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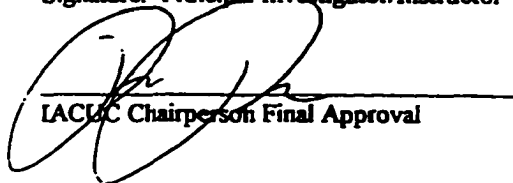
To ensure that my study is not
duplication, I made a thorough search of
the Medline database (1983 - present), as well
as the most inclusive database available
to the field of Behavior & Pharmacology.
- Jan Hirst

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Acceptance of Provisions



Signature: Principal Investigator/Instructor

7/28/97
Date

LACUC Chairperson Final Approval

8/6/97
DateApproved LACUC Number 97-06-02

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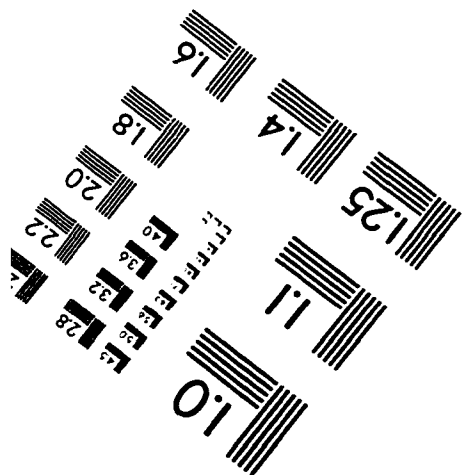
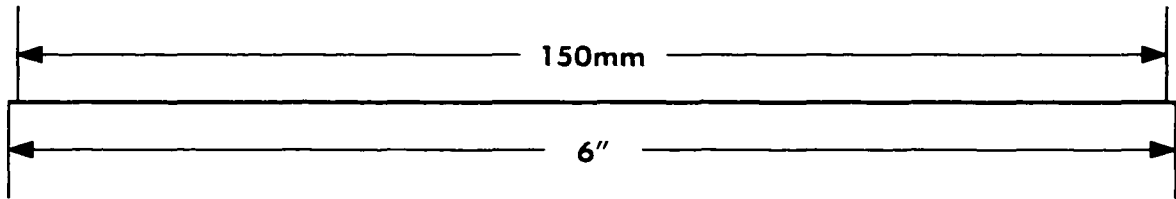
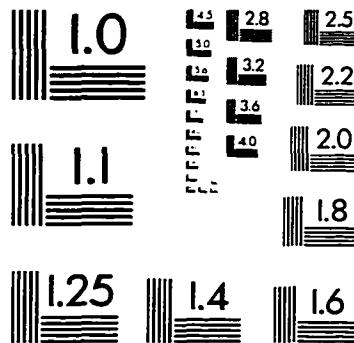
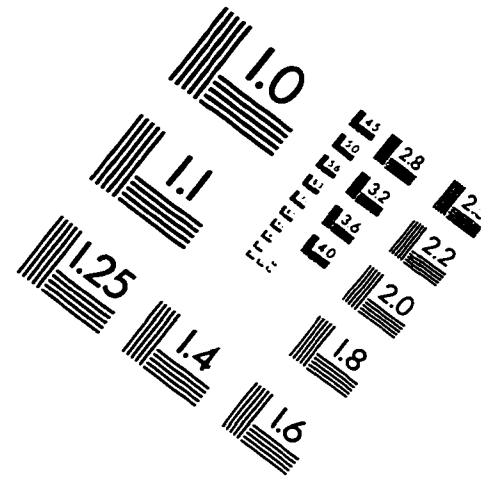
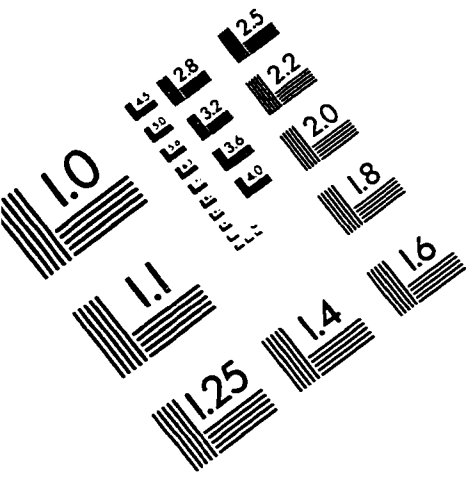
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IMAGE EVALUATION TEST TARGET (QA-3)



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