8-1998

MDMA and Learning: Effects of Acute and Neurotoxic Exposure

Thomas P. Byrne
Western Michigan University

MDMA AND LEARNING: EFFECTS OF ACUTE AND NEUROTOXIC EXPOSURE

by

Thomas P. Byrne

A Dissertation
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Doctor of Philosophy
Department of Psychology

Western Michigan University
Kalamazoo, Michigan
August 1998

MDMA AND LEARNING: EFFECTS OF ACUTE AND NEUROTOXIC EXPOSURE

Thomas P. Byrne, Ph.D.

Western Michigan University, 1998

In two experiments, the effects of MDMA were examined on the acquisition of lever-press responding by rats exposed to procedures in which water delivery was delayed by 0, 10, or 20 seconds. In the first experiment, naive, water-deprived rats received an intraperitoneal injection of MDMA (0, 1.0, 3.2, or 5.6 mg/kg) prior to one experimental session. Response acquisition was observed under all conditions at all drug doses. MDMA increased both response rates and reinforcers earned in a dose-dependent fashion, but only when reinforcement was immediate. Under conditions of delay, MDMA had no effect on the number of lever presses emitted or reinforcers earned. Under all reinforcement conditions, higher doses of MDMA typically produced an initial reduction in lever pressing followed by accelerated rates of responding. In the second experiment, rats received an MDMA injection regimen previously shown to be neurotoxic. Control rats received saline solution according to the same injection schedule. Two weeks after completing the regimen, rats were water-deprived and exposed to behavioral procedures as described for the first experiment. Performance of rats exposed to MDMA did not differ from that of rats exposed to saline. Thus, in both experiments, MDMA failed to disrupt learning.

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ACKNOWLEDGMENTS

First and foremost, I would like thank the members of my committee. Dr. Alan Poling, Dr. Lisa Baker, Dr. Jack Michael and Dr. Morton Wagenfeld have each contributed significantly to my education. My graduate experience at Western Michigan University has exceeded all expectations, and each of my committee members has been an important part of that experience.

I would like to extend special appreciation to my advisor, Alan Poling. He has truly been a great teacher. His dedication to his students is exceptional, and I have benefited immensely because of it.

My support network has been phenomenal. I owe a great deal to my wife, Serah, who has sacrificed much so that I could concentrate on scholarship. Her friendship has been invaluable. Finally I would like to thank my parents, Kathy and Tom, as well as my brother, Chris, for years of love and support.

Thomas P. Byrne

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CHAPTER I

INTRODUCTION

Response Acquisition With Delayed Reinforcement

In the experimental analysis of behavior, there has been a recent increase in the number of studies examining the acquisition of new, operant behavior. Increased interest in acquisition was galvanized by Lattal and Gleeson (1990), who showed that naïve organisms can acquire new operant behavior in the absence of any direct shaping or autoshaping and when reinforcement was delayed by up to 30 seconds. In a series of experiments, rats and pigeons acquired lever-pressing and key-pecking, respectively, over 20 to 25 daily experimental sessions in which there were no immediate programmed consequences for responding. These data called into question long-held beliefs that reinforcement must be immediate in order to be effective (Lattal & Williams, 1997). Since Lattal and Gleeson's initial study, investigators using similar procedures have demonstrated response acquisition with delayed reinforcement using various delays (Dickinson, Watt & Griffiths, 1992; Sutphin, Byrne & Poling, 1996; van Haren, 1992;Wilkenfield, Nickle, Blakely, & Poling, (1992), responses (Critchfield & Lattal, 1993; Schlinger & Blakely, 1994), and species (Lattal & Metzger, 1994).

The study by Wilkenfield et al. (1992) is noteworthy for two reasons. First,

unlike the repeated sessions used by Lattal and Gleeson, Wilkenfield et al. exposed subjects to one eight-hour test session. Such a procedure allowed for an examination of acquisition in an efficient manner. Second, Wilkenfield et al. compared lever-press acquisition in rats under three different procedures for arranging reinforcement delays and provided a detailed analysis of the difficulties associated with each procedure. One procedure, the nonresetting delay, consisted of a fixed-ratio (FR) 1 fixed-time (FT) *t* schedule of reinforcement. Under this arrangement, the initial press of a lever and each subsequent first press after reinforcer delivery initiated a delay interval which was followed by food delivery. Presses during the delay interval had no programmed consequences. Two problems are associated with this procedure. First, because presses during the delay have no programmed consequences, there is no direct relation between rates of reinforcement and rates of responding. Second, the obtained delays can be less than nominal delays under this procedure.

Another type of procedure described by Wilkenfield et al., the stacked delay, does ensure a direct relationship between response rates and reinforcement rates. Here, each and every lever-press started an FT interval followed by reinforcer delivery. For example, under a stacked delay of 10 s, responses emitted at 1, 5, and 10 seconds into the session produced reinforcer deliveries at 11, 15, and 20 seconds into the session. However, under such an arrangement obtained delays may be shorter than programmed delays. In the Wilkenfield et al. study, average obtained delays were in fact shorter that programmed delays for both the stacked and nonresetting procedures.

The third delay procedure examined by Wilkenfield et al. was a resetting delay. Here, rats were exposed to a tandem FR 1 not-responding-greater-than t (R >t) schedule of reinforcement. Under this schedule, the first press of the lever started a delay interval of t seconds, after which food was delivered. Any presses that occurred before the termination of the interval restarted the delay. This procedure guarantees that obtained and programmed delays are equal. However, there is no direct relation between response rates and reinforcement rates. Furthermore, increasing response rates such that interresponse times (IRT's) are shorter than the programmed delay interval will decrease reinforcement rates. Unsurprisingly, such a contingency can generate low rates of responding (Dews, 1960; LeSage, Byrne, & Poling, 1996; Sutphin et al., 1998). Although Wilkenfield et al. demonstrated response acquisition with delayed reinforcement under each of the three procedures at delays of 4-, 8- and 16-s, the rate and temporal pattern observed depended critically on the procedure used.

Behavioral pharmacology has borrowed heavily from the methodology used in the experimental analysis of behavior. Because most studies in the experimental analysis examine steady state behavior, it is unsurprising that the majority of investigations conducted by behavioral pharmacologists have been studies of drug effects on steady states of behavior. Drug effects on behavior in transition, including the acquisition of new operant behavior, has received comparatively little attention (Branch, 1977; Commons, Woodford, Boitano, Ducheney, & Peck, 1982; LeSage et al., 1996).

Although there are numerous studies examining the effects of drugs on learning, the most common assay used is the repeated acquisition procedure which employs steady sates of responding as a baseline against which drug effects are probed (Paule & McMillan, 1984). The specific responses involved, for example key pecks by pigeons or lever presses by rats, are acquired by the subjects long before drug effects are examined.

As Branch (1991) has pointed out, behavioral pharmacologists may profit by focusing more effort on studying behavior in transition. This advice may be especially relevant for studying behavior acquired with delayed reinforcement. It is generally accepted that learning occurs less readily when reinforcement is delayed than when it is immediate (Johnston & Pennypacker, 1993; Skinner, 1953). Given this, and the characteristic observation that poorly-learned behaviors are more easily disrupted by drugs than are better-learned behaviors (Byrne, LeSage &Poling, 1997; Picker & Negus, 1993; Poling, 1986), it is reasonable to expect that a given drug and dose would interfere with the initial acquisition of operant behavior to a greater extent when reinforcement is delayed than when it is immediate. Therefore, response acquisition procedures similar to those used by Wilkenfield may produce behavior that is sensitive to drug effects and provide a reasonable methodology for detecting those effects.

The first paper reporting the effects of drugs on acquisition with delayed reinforcement was published by LeSage, Byrne, and Poling in 1996. The authors used behavioral procedures identical to those used by Wilkenfield, with the exception

that water, not food, was used for reinforcement, and the stacked delay procedure was not utilized. In a between-subjects design, naive rats were given intraperitoneal (IP) injections of 0, 1.0, 5.6, or 10.0 mg/kg *d*-amphetamine and exposed to resetting or nonresetting delays of 0, 8, or 16 seconds. Rats acquired lever pressing under all dose and delay parameters. Although delay did not modulate the effects of *d*-amphetamine as had been hypothesized, the assay allowed for the detection of, dose-dependent effects of *d*-amphetamine on operant behavior. Small doses had no effect or increased behavior; larger doses induced stereotypy at the beginning of the session followed by accelerated responses rates. These effects are consistent with the results of *d*-amphetamine under repeated acquisition procedures where *d*-amphetamine does not interfere with learning unless doses used are large enough to produce general suppression of behavior (Evans & Wenger, 1992; Paule & McMillan, 1984; Thompson, 1974).

Like Wilkenfield et al., LeSage et al. used two response levers. One lever, termed the operative lever, produced reinforcement according to the assigned schedule. The second lever, termed the inoperative lever, had no programmed consequences. As stated by LeSage et al., the development of differential responding on the two levers can be considered as a measure of sensitivity to programmed consequences as well as a measure of response efficiency. Essentially, if a greater number of responses are emitted on the operative lever, then it is fair to say that the lever that produces reinforcement exercises greater stimulus control over responding. If this occurs, the organism could be said to be sensitive to the consequences of its

behavior. In general, LeSage et al. found no effect of *d*-amphetamine on the development of differential responding.

However, in a follow-up study by Byrne et al. (1997), chlorpromazine was tested under similar procedures, and drug effects on differential responding were evident. Interestingly, chlorpromazine affected the development of differential responding under conditions of delayed reinforcement only. Naive rats were given IP injections of 0, 2, 6, and 10 mg/kg chlorpromazine and exposed to conditions of immediate reinforcement or nonresetting reinforcement delays of 8 seconds. Acquisition occurred for all rats except for some exposed to 10 mg/kg, which suppressed all behavior. For rats exposed to delayed reinforcement, chlorpromazine at 2 and 6 mg/kg produced a dose-dependent increase in the percentage of lever-pressing allocated to the inoperative lever. This did not occur under conditions of immediate reinforcement. These results support the possibility that delaying reinforcement may allow for the detection of drug effects not apparent when reinforcement is immediate.

Methodological Improvements

A study by Sutphin, Byrne, and Poling (1998) demonstrated significant methodological improvements in the response acquisition procedure. Because the behavioral procedures used by Sutphin et al. were adopted for the present study, they will be presented here in detail.

As discussed by Sutphin et al., resetting delay procedures are perhaps the best way to arrange reinforcement delays when examining acquisition as they guarantee

that nominal and obtained delays are equivalent. However, as mentioned above, rate of responding is not a good index of sensitivity to programmed contingencies under such arrangements. $\overline{R} > t$ schedules reduces response rates of established operants, and the magnitude of the response reduction is directly related to the length of t (Zeiler, 1971; Zeiler, 1976, Zeiler, 1979). At long delays, subjects are exposed to both a powerful response-reducing contingency and to delayed reinforcement. It is therefore not surprising that prior studies have shown that at 16 and 32 s resetting delays, rats emitted as many, or more responses on an inoperative lever as on an operative lever (LeSage et al., 1996; Wilkenfield et al., 1992).

One interpretation of these findings is that the rats' behavior was not sensitive to its consequences at these delay values. Rats typically explore novel environments, and occasional lever presses are likely to occur in the confined environment of an operant chamber for reasons other than previous reinforcement history. Such presses will produce a programmed reinforcer (food or water). The activity level of deprived animals that occasionally receive food (or water) may increase, and lever-pressing may thereby be more likely even though no process of reinforcement is involved. If this occurred, rats should emit similar rates of responding on the two. This possibility does not appear likely, given that Dickinson et al. (1992) and LeSage et al. (1996) observed little lever pressing by yoked-control rats that received food whenever food was earned under nonresetting delay procedures by master partners and for which lever presses had no programmed consequences.

Another interpretation was offered by Wilkenfield et al. (1992), who proposed

that both operative-lever and inoperative-lever responses were strengthened by food delivery. Reinforcement was response independent in the latter case, but under the resetting procedure delays were nonetheless shorter for inoperative-lever responses. Moreover, the $\bar{R} > t$ contingency was incompatible with high rates of responding on the operative lever and may have indirectly increased the likelihood of behavior incompatible with pressing the operative lever. One such behavior would be pressing the inoperative lever. For these reasons, it is not surprising that operative-lever responding did not exceed inoperative-lever responding at some delays.

Comparing levels of responding on operative and inoperative levers obviously does not provide an uncontaminated index of sensitivity to reinforcement contingencies under resetting delay procedures. To provide a better index of such sensitivity, Sutphin et al. (1998) examined procedures in which responses on one lever, the reinforcement lever, produced water after a resetting delay of 8, 16, 32, or 64 s, and responses on a second lever, the cancellation lever, prevented any scheduled water delivery. Therefore, responses on this lever never produced water and were never followed by water by a delay shorter than that programmed on the operative lever. To determine whether this comparison would yield results different from those obtained when responses on an operative and inoperative lever are compared, the study also arranged 8-, 16-, 32 and 64-s resetting delays under conditions similar to the studies by LeSage et al. (1996) and Wilkenfield et al. (1996). At delays of 16 and 32 s there was clear evidence of differential responding on the two levers under the cancellation condition, but not under the inoperative-lever

condition. In sum, results from the Sutphin et al. study (1998) suggest that the resetting/cancellation procedure provides unambiguous evidence that consequences delayed by up to 30 s affect behavior.

Because the resetting/cancellation procedure guarantees the equivalence of nominal and obtained delays, eliminates the possibility of adventitious reinforcement on the lever that does not produce reinforcement, and engenders response acquisition with substantial reinforcement delays, it shows promise for examining the modulation of drug effects on learning. Such a procedure was used in the present investigation to study the effects of MDMA.

An MDMA Primer

The drug (±) 3,4-methylenedioxymethamphetamine (MDMA) is a compound structurally similar to both the hallucinogen mescaline and the amphetamines (Baker & Taylor, 1997; Schecter, 1998). Although it was first patented by Merck in 1914 (Steele, McCann, & Ricuarte, 1994), it did not receive much attention until it came into use as both a recreational drug and a therapeutic agent used in conjunction with psychotherapy in the 1970s.

MDMA was adopted for use in clinical settings due to its reported ability to increase empathy and strengthen the relationships between therapists and their clients (Green, Cross, & Goodwin, 1995; Greer & Tolbert, 1986). Probably coincidentally, the therapeutic interest in the drug coincided with an increase in its recreational use. Known by the slang names of "Ecstasy", "Adam", or "E", MDMA became popular

within the European dance club rave culture, which soon spread to the United States. Ruling that the drug had no established medical benefits, the Drug Enforcement Agency temporarily assigned MDMA as a Schedule 1 substance in 1985. Shortly thereafter, an influential study published in *Science* suggested that MDMA may damage serotonergic neurons (Ricuarte, Bryan, Strauss, Seiden, & Schuster, 1985). These findings were replicated, which contributed to the Drug Enforcement Agency permanently assigning MDMA to Schedule 1 status in 1986 (Lawn, 1986).

Studies examining behavioral and pharmacological effects of MDMA in humans have been severely limited due to its Schedule 1 status and reports of neurotoxicity. Therefore, most of what is known about its pharmacology and behavioral effects has been derived from nonhuman research and clinical observations made before the substance was restricted. O'Brien (1996) reports that, "Acute effects [in humans] are dose dependent and include tachycardia, dry mouth, jaw clenching, and muscle aches. At higher doses, the effects include visual hallucinations, agitation, hyperthermia, and panic attacks "(p. 574). Positive subjective reports of the effects of MDMA typically include increased empathy, energy, and feelings of well-being (Saunders & Doblin, 1996). When used recreationally, MDMA is usually administered orally in doses typically ranging from roughly 50 to 125 mg (Saunders & Doblin, 1996) and has a half-life of about seven hours (Alrazi & Vereby, 1988).

Studies in nonhumans have shown that MDMA acts on both serotonergic and dopaminergic systems (e.g., Schecter, 1989, 1998), although the effects on serotonergic systems seem to be more prominent (Steele et al., 1994). MDMA

releases serotonin (5H-T) from presynaptic terminals and interferes with its re-uptake (Lim & Foltz, 1988; Nash, Meltzer, & Gudelsky, 1990). In rats, MDMA is metabolized primarily in the liver (Lim & Foltz, 1988), and the half-life has been reported at 73.8 min for (+)MDMA and 100.7 min for (-) MDMA (Cho, Hiramatsu, DiStefano, Chang, & Jenden, 1990). At least eight metabolites of MDMA have been identified, including 3,4-methylenedioxyamphetamine (MDA); most are excreted in urine (Lim & Foltz, 1988).

With certain regimens, MDMA reduces levels of serotonin in the brains of rats and nonhuman primates (Green et al., 1995). Measurable damage to serotonin axons has also been reported (Molliver et al., 1990). However, the mechanisms by which MDMA functions as a neurotoxin are not completely understood at this time (McCann & Ricaurte, 1993; Nash et al., 1990). There is some evidence that neurotoxicity may involve interactions of dopaminergic and serotonergic systems, because depletion of dopamine stores prior to MDMA administration counteracts MDMA-induced depletion of serotonin (Stone, Johnson, Hanson, & Gibb, 1988).

Despite law enforcement efforts and mounting evidence of its neurotoxicity in nonhumans and possible neurotoxic effects in humans (Ricuarte, Finnegan, Irwin, & Langston, 1990), recreational use of MDMA continues. One study (Cuomo, Dyment, & Gammino, 1994) found that the number of college students who had used MDMA recreationally increased from 16 % to 24% between 1986 and 1990, a period in which many reports of potential neurotoxicity were disseminated. However, the fact that MDMA has become a drug of abuse may be unsurprising considering that it functions

as a positive reinforcer for nonhumans in self-administration assays (Beardsly, Balster, & Harris, 1986; Lamb & Griffiths, 1987). Despite its popularity in the scientific literature and the media, MDMA does not seem to present a major public health problem. Medical complications and overdose from MDMA have been rare (Lim & Foltz, 1988), although they have been reported (Creighton, Black, & Hyde, 1991; Dowling, McDonough, & Bost, 1987; Henry, Jeffreys, & Dawling, 1992). As discussed below, there is little or no evidence that MDMA-induced neurotoxicity causes any serious behavioral disruption (Saunders & Doblin, 1996). Furthermore, survey data indicate that rates of MDMA use are below those for alcohol, nicotine, phencyclidine, amyl nitrate, marijuana, mescaline, cocaine, methamphetamine, and LSD (Substance Abuse and Mental Health Services Administration, 1996).

Although current problems caused by the recreational interest in MDMA seem minor compared to those associated with other drugs of abuse, scientific interest in the compound has been exceptional. It appears that this interest arises, in large part, from the drug's toxic effects on serotonergic systems. In addition, the drug has some unique properties. In particular, its subjective effects share similarities with both stimulants and hallucinogens as revealed by verbal reports in humans and drug discrimination studies with nonhumans (Baker & Taylor, 1997; Gold, Koob, & Geyer, 1988; Oberlender & Nichols, 1988). Primarily for this reason, some scientists have proposed that MDMA and related compounds (MDA, MBDB, MDEA) belong to a separate drug class called entactogens (see Nichols, 1986).

MDMA and Learning

Although interest in MDMA has generated a substantial literature over the past several years, the primary focus of inquiry has been the neurotoxic capacity of the drug (Steele et al., 1994), and investigations of its behavioral effects have been limited (Frederick et al., 1995a; LeSage, Clark, & Poling, 1993). In particular, there have been few studies concerning the effects of MDMA on learning and memory, and no reports of the drug's effect on the acquisition of new behavior.

Examinations of possible effects of MDMA on repeated acquisition and delayed-matching-to-sample procedures have yielded inconclusive results. Thompson, Winsauer, and Mastropaolo (1987) examined the acute effects of MDMA in patas monkeys performing under a repeated acquisition procedure and found that the drug did not affect acquisition of response chains. In contrast, Frederick, Gillam, Allen, and Paule (1995b) reported that acute administrations of MDMA increased errors in rhesus monkeys performing under an incremental repeated acquisition procedure at doses that did not disrupt response rates. Frederick et al. (1995b) also found that MDMA did not affect performance under a delayed-matching-to-sample (DMTS) procedure. This finding is inconsistent with the results of a study by LeSage Clark, and Poling (1993), who found that MDMA, when acutely administered, reduced DMTS accuracy in pigeons.

There is very little information concerning the chronic effects of MDMA on learning and memory. The neurotoxic effects of repeated, high-doses of MDMA

administration have been repeatedly and convincingly demonstrated in both rats (e.g., Commins et al., 1987; Ricuarte et al., 1985; Ricuarte et al., 1993; Stone, Stahl, Hansen, & Gibb, 1986) and non-human primates (Ali et al., 1993; Klevin, Woolverton, & Seiden, 1989; Ricaurte et al., 1988). It appears, however, that exposure to a neurotoxic MDMA regimen usually fails to disrupt behavior (Frederick et al., 1995a; Ricuarte et al., 1993), although exceptions have been reported (Ali, Scallet, Holson, Newport, & Slikker, 1987 ; Schecter, 1991). In fact, with chronic exposure tolerance develops to the drug's disruptive effects under DMTS and repeated acquisition procedures (Frederick at el, 1995a; LeSage et al., 1993). Currently, there is no evidence that MDMA-induced neurotoxicity affects learning.

The Experimental Questions

The present investigation used the assay developed by Sutphin et al. (1998) to examine the effects of acute and neurotoxic exposure to MDMA on response acquisition with immediate and delayed reinforcement in rats. The assay was chosen for three reasons. First, the procedure appears useful for analyzing the initial acquisition of operant behavior as measured by accelerated rates of lever-pressing as well as by differential responding on two levers, each associated with either reinforcing or response-reducing contingencies. Second, this assay may produce behavior that is especially sensitive to drug effects. Previous work suggests that delaying reinforcement during acquisition may allow for detection of drug effects not apparent with immediate reinforcement (Byrne et al., 1997). Finally, MDMA has

some stimulant-like properties (Baker & Taylor, 1997; Kamien, Johanson, Schuster, & Woolverton, 1986; Oberlender & Nichols, 1988; Schecter, 1998), and it is interesting to compare the effects of MDMA to the effects of *d*-amphetamine under similar procedures (LeSage et al., 1996). Experiment 1 was designed to examine the acute effects of MDMA on learning, and Experiment 2 was designed to examine the effects of chronic MDMA-induced neurotoxicity on learning.

CHAPTER II

EXPERIMENT 1

Materials and Methods

Subjects

Ninety-six experimentally-naïve male Sprague-Dawley rats, approximately 60 to 70 days of age, served as subjects. The rats were water deprived as described below and were housed individually with unlimited access to food in a colony area with a 12-hr light/dark cycle.

Apparatus

Eight MED Associates (St. Albans, VT) operant test chambers were used. The chambers were 28 cm long by 21 cm wide by 21 cm high. During response-acquisition sessions, two response levers separated by 8.5 cm were mounted on the front panel 7 cm above the chamber floor. The levers were removed during dipper training sessions. A minimum force of 0.14 N was required to operate the levers. A receptacle, located in the center of the front panel 3 cm above the chamber floor, allowed access to a dipper filled with 0.1 ml of tap water. Chambers were illuminated by a 7-watt bulb mounted on the ceiling. An exhaust fan in each chamber masked

extraneous noise and provided ventilation. Programming of experimental events and data recording were controlled by an IBM-compatible microcomputer equipped with MED-PC software.

Behavioral Procedure

All subjects were water deprived for 24 hours prior to one dipper training session. Dipper-training sessions were 90 min in length. During dipper-training sessions water was delivered under a variable-time 60-s schedule. Under this schedule, 4-s dipper presentations occurred randomly on average once every 60-s, regardless of the subject's behavior. All rats were observed to drink from the dipper by the end of the session. At the end of dipper training sessions rats were returned to their home cages and given 20 min access to water.

Twenty-four hours after dipper training subjects were exposed to one response-acquisition session. Response-acquisition sessions began at approximately 10:00 p.m. and lasted for 8 hr. Rats were randomly assigned to 12 acquisition conditions (squads), with eight rats in each squad. Subjects were exposed to a tandem FR 1 $\bar{R} > t$ schedule on the reinforcement lever. Here, the first response initiated a delay of t s, after which water was delivered for 4 s. Values of t were 0, 10, and 20 s for various groups. There were four squads exposed to each delay value. The left lever was designated as the reinforcement lever for four randomly-selected rats in each squad, and the right lever was designated as the reinforcement lever for the remaining rats. If a response occurred on the other (cancellation) lever during a delay

(t) interval, the scheduled water delivery did not occur. Responses on this lever at other times had no scheduled consequences, but were recorded. For subjects that received immediate 4-s water deliveries ($t = 0$ s) after pressing the reinforcement lever, responding on the second lever had no consequences but were recorded. Because previous reports have demonstrated that no-water and yoked-control subjects emit considerably less responding than rats exposed to delay values equal to or greater than those utilized in this study (Dickinson et al., 1992; LeSage et al., 1996; Sutphin et al., 1998), no such controls were used in the present investigation.

Under all conditions, responses on the two levers were recorded in 5-min bins across the course of the session. Total water deliveries during each session were also recorded.

Pharmacological Procedure

Squads of rats from each delay value were injected once with 0, 1.0, 3.2 or 5.6 mg/kg of MDMA (National Institute on Drug Abuse, Rockville, MD). All injections were given IP 15 min prior to the start of the response-acquisition sessions. Rats were placed in operant chambers five minutes prior to session commencement. The drug was dissolved in 0.85% saline solution and prepared at an injection volume of 1ml/kgDoses and presession injection times were based on pilot data from our laboratory.

Results

All subjects, except for one rat in the 5.6 mg/kg, 20-s delay group, emitted accelerated rates of pressing on the reinforcement lever and thus showed clear evidence of response acquisition. Data from one rat in the 3.2 mg/kg 10-s group were lost over the course of the session due to equipment failure. Figure 1 shows mean cumulative reinforcement-lever responses (acquisition curves) for each group. In general, increasing delay of reinforcement decreased responding on the reinforcement lever. However, for groups injected with vehicle, there appears to be an equal amount of responding on the reinforcement lever across all delays if total cumulative responses at the end of the session are compared. This is not the case for rats exposed to MDMA, for which total cumulative responses on the reinforcement lever were higher in groups exposed to immediate reinforcement. This is most evident in rats that received 5.6 mg/kg.

As shown in Figure 1, rats injected with vehicle or 1.0 mg/kg MDMA started responding soon after the session commenced. MDMA at doses of 3.2 mg/kg and 5.6 mg/kg displaced the start of accelerated responding (i.e. rats began responding later in the session), although subjects responded at rates comparable or higher than vehicle-control rates once responding began. Most subjects injected with 5.6 mg/kg did not start responding until about 100 min into the session. Individual data for all subjects are depicted in the appendix. Casual observation indicated that the subjects injected with 5.6 mg/kg exhibited a general depression of behavior during the beginning of the

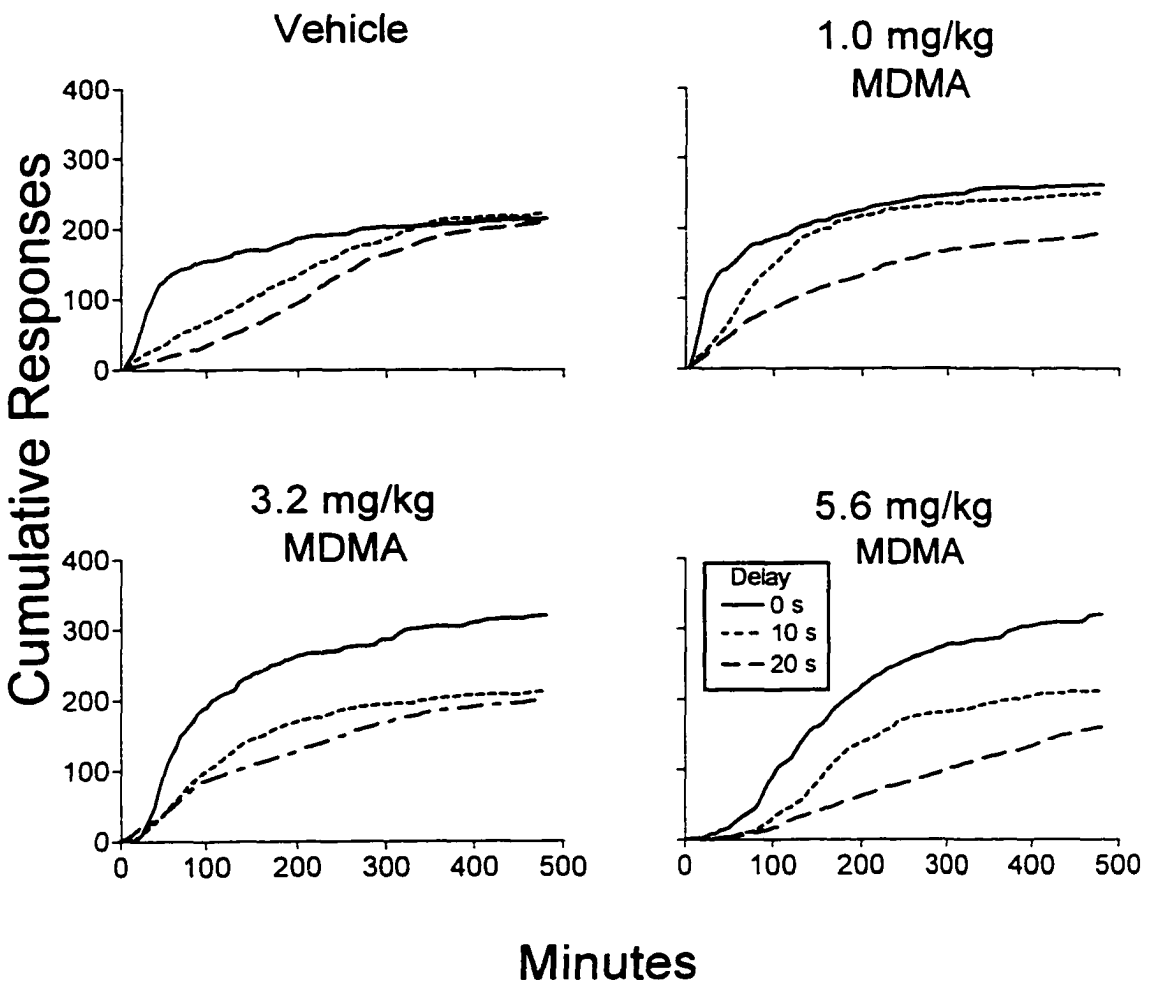


Figure 1. Mean Cumulative Reinforcement-Lever Responses Under Each Experimental Condition.

session and did not emit exploratory behavior as seen in rats injected with vehicle or lower MDMA doses.

Figure 2 depicts mean total responses and mean total reinforcers for each group. For rats exposed to immediate reinforcement, MDMA increased the number of reinforcement-lever responses and resulting water deliveries in a dose-dependent fashion. However, for groups exposed to delays of 10 or 20-s, MDMA did not affect the total number of reinforcement-lever responses or reinforcers earned. Analysis by two-way ANOVA showed a significant interaction of the effects of drug and delay on total reinforcement-lever responding ($F = 3.22, p < .01$), suggesting that reinforcement delay modulated the effects of MDMA. Such an interaction makes the effects of drug or delay alone difficult to interpret. When the effect of drug is analyzed across all delay values, there is not a significant effect on total reinforcement-lever responses ($F = .98, p = .47$). However, as Figure 2 shows, there were marked differences in mean reinforcement-lever responses between squads exposed to various doses of MDMA when reinforcement was immediate but not when reinforcement was delayed. For this reason, data from squads exposed to immediate reinforcement were analyzed separately. When this was done, differences among doses in mean reinforcement-lever responding were significant ($F = 7.45, p < .01$). Multiple comparisons with a Tukey HSD show significant differences in reinforcement-lever responding between vehicle and 3.2 mg/kg ($q = 5.65, p < .01$), as well as vehicle and 5.6 mg/kg ($q = 5.63, p < .01$). Comparisons between all other groups exposed to immediate reinforcement are not significant.

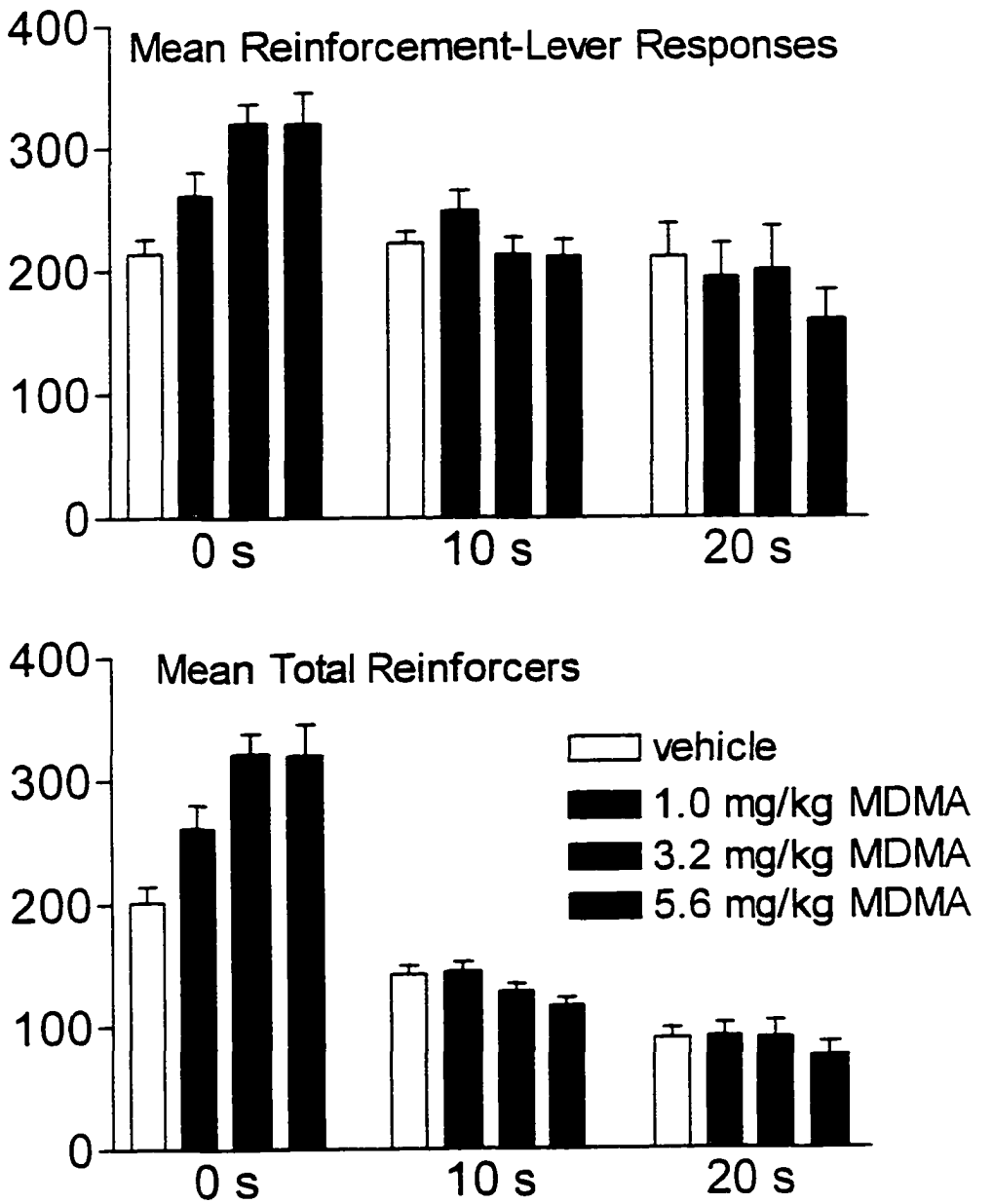


Figure 2. Mean Total Reinforcement-Lever Presses and Total Reinforcers Earned Under Each Experimental Condition.

Figure 3 was constructed for visual analysis of the dynamics of responding at the lowest and highest parameters of drug and delay. As shown in the top panel, rats exposed to vehicle injections and immediate reinforcement responded at high rates early in the session, and acceleration of responding slowed considerably about 50-60 min into the session. Rats exposed to 5.6 mg/kg MDMA and immediate reinforcement responded slowly at the beginning of the session, but response rates never decelerated as quickly as for rats exposed to vehicle. Although lever presses are depicted, there is a direct correspondence between presses and reinforcer deliveries under immediate reinforcement conditions. Therefore, the top panel is a reasonable depiction of cumulative reinforcer deliveries. The point of decelerated responding may indicate satiation for rats exposed to vehicle at a mean of roughly 150 water deliveries. On average, however, rats exposed to 5.6 mg/kg did not show such deceleration after receiving the same number of water deliveries. The bottom panel depicts group data for rats exposed to a 20-s reinforcement delay. Here, deceleration is not evident, suggesting rats may not have earned enough reinforcers during the session to become satiated.

A second measure of learning, differential responding on the reinforcement and the cancellation levers, is shown in Figure 4, which depicts mean proportion of reinforcement- to cancellation-lever responding for each group. Most subjects emitted the majority of lever presses on the reinforcement lever. MDMA ($F = .50, p = .68$) and delay length ($F = 2.82, p = .06$) did not significantly affect the proportion of responses controlled by the reinforcement-lever. However, all rats in the

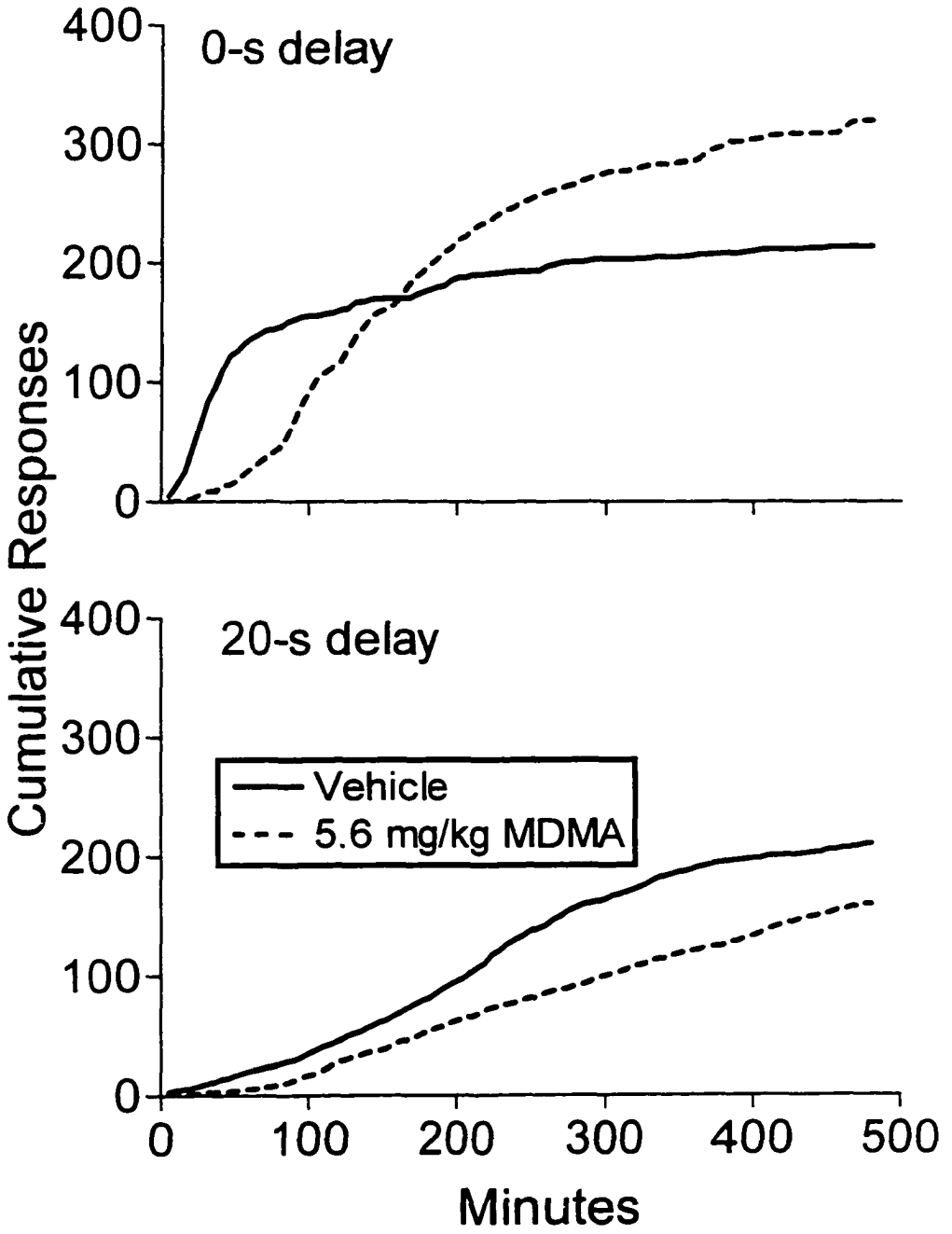


Figure 3. Effects of 5.6 mg/kg MDMA on Mean Cumulative Responses by Rats Exposed to 0-s or 20-s Delays.

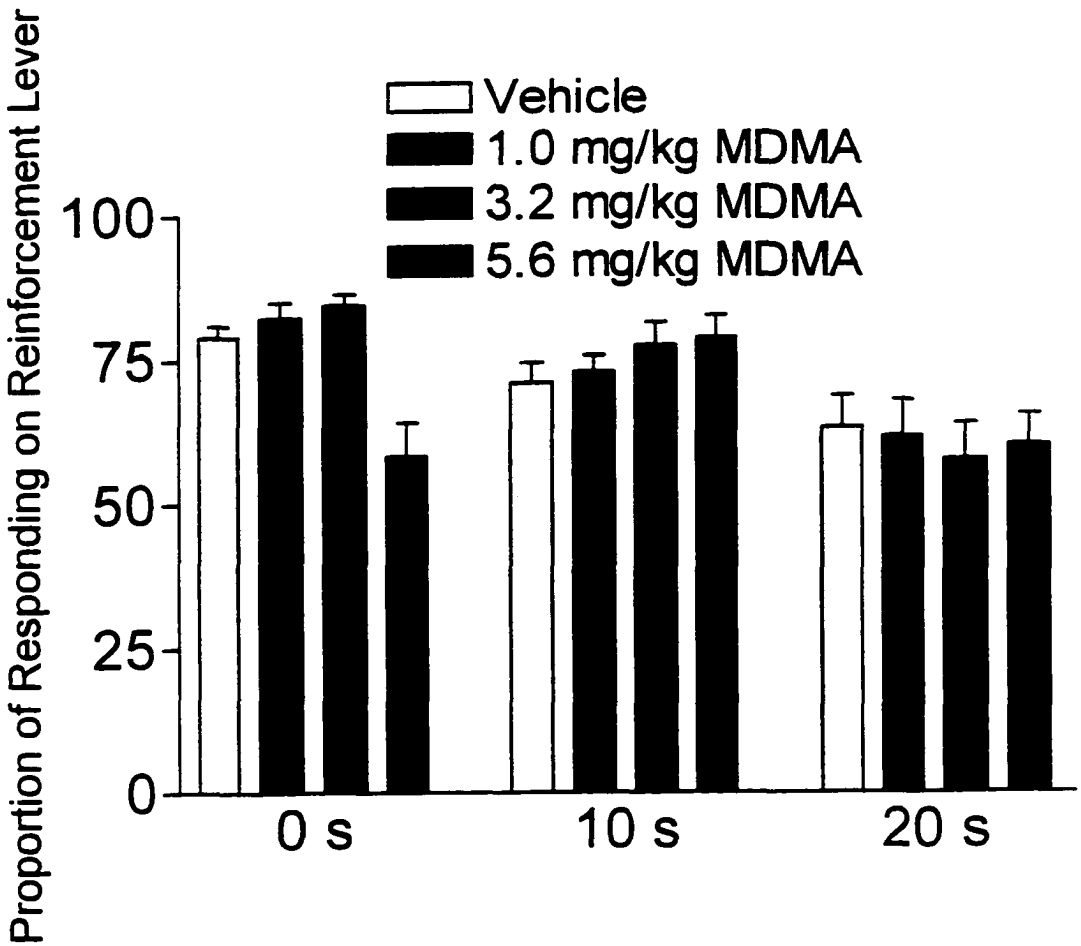


Figure 4. Mean Proportion of Reinforcement-Lever Responses.

immediate-reinforcement and 10-s delay groups emitted more responses on the reinforcement lever than on the cancellation-lever. Four rats exposed to 20-s delays, one rat each from the vehicle and 5.6 mg/kg groups and two rats from the 1.0 mg/kg group, emitted more responses on the cancellation lever.

Discussion

Under conditions of immediate reinforcement, MDMA increased reinforcement-lever responding and reinforcement rates, and therefore enhanced acquisition. These findings are similar to those found by LeSage at el. (1996) with *d*-amphetamine under comparable procedures. Those authors reported that *d*-amphetamine increased overall rates of operative-lever responding in rats under resetting delay procedures. However, the rate-increasing effect of *d*-amphetamine was seen when reinforcement was both immediate and delayed; this differs from the rate-increasing effects of MDMA in the present study, which were only evident when reinforcement was immediate.

Doses of 3.2 mg/kg and 5.6 mg/kg MDMA suppressed acquisition of responding at the beginning of the session. Similar results were found by LeSage et al., with 5.6 and 10.0 mg/kg *d*-amphetamine. These doses produced stereotypy (sniffing and licking the chamber flour) incompatible with lever pressing, and few presses occurred for the first 100 min or longer of the session. Although similar suppression was evident with 3.2 and especially 5.6 mg/kg MDMA, stereotypy was not observed.

MDMA did not affect responding on the cancellation lever. Likewise, LeSage et al. reported that *d*-amphetamine did not affect responding on an inoperative lever. As discussed above, the inoperative lever differed from the cancellation lever in that responses on the inoperative lever had no consequences. Both levers are employed to test for the development of differential responding, which demonstrates sensitivity to reinforcement. MDMA, like *d*-amphetamine, does not appear to interfere with the development of differential responding with immediate or with delayed reinforcement. This differs from results with chlorpromazine which interfered with the development of differential responding, but only when reinforcement was delayed (Byrne et al.1997).

Why MDMA increased reinforcement-lever responding under conditions of immediate reinforcement and not delayed reinforcement is unclear. MDMA has been shown to increase locomotor activity (Gold et al., 1988; Spanos & Yamamoto, 1989; Steele et al., 1994), and it is possible that it did so in the present study. Under conditions of immediate reinforcement, any behavior resulting in a "lever-press" would result in reinforcement regardless of response rates. Therefore, an increase in general activity and lever-pressing induced by MDMA would likely increase reinforcement rates. However, under conditions of delay, responding at rates higher than those allowed by the $\bar{R} > t$ contingency would reset the delay interval, so a greater overall increase in behavior would not necessarily produce greater reinforcement rates. Furthermore, other behaviors that occurred in close temporal proximity to water delivery could be adventitiously reinforced. Such responses would

essentially compete with lever pressing.

Interestingly, Gold et al. (1988) demonstrated that rats injected with MDMA exhibited a tendency to limit locomotion to the perimeter of experimental chambers. If such behavior occurred in the present study, it would increase the probability of initial lever contact and enhance acquisition in the immediate reinforcement group. MDMA also produces hyperthermia (Steele et al., 1994), which may have increased the reinforcing effectiveness of water. Such an analysis is supported by Figure 3 which shows that rats exposed to vehicle responded at slower rates than rats exposed to 5.6 mg/kg MDMA at times when both groups had received the same number of reinforcers. However, it is not clear why such an establishing operation (see Michael, 1993) would not improve performance with delayed reinforcement as well. It may be that MDMA did not so much enhance acquisition per se, but simply extended the period of time in which established lever-pressing was effectively reinforced by water.

The effects of drugs on behavior in transitional states have received relatively little attention (Branch, 1991), as have the conditions necessary for response acquisition. The acquisition of new behavior as well as the effects of delayed reinforcement may be quite complex, and it appears that arranging delays of reinforcement can either amplify or diminish the effects of drugs on acquisition.

CHAPTER III

EXPERIMENT 2

Material and Methods

Experiment 2 was designed to examine whether a neurotoxic regimen of MDMA affected the acquisition of new behavior.

Subjects

Forty-eight experimentally-naïve-male-Sprague-Dawley rats, approximately 60 to 70 days of age at the start of the experiment, served as subjects. The rats were housed and water-deprived as described in Experiment 1.

Apparatus

The apparatus was identical to that in Experiment 1.

Pharmacological Procedure

Rats were randomly assigned to MDMA or vehicle dosing regimens. Twenty-four rats received subcutaneous injections of 20 mg/kg MDMA (National Institute on Drug Abuse, Rockville, MD) twice a day (8:00 a.m. and 8:00 p.m.) for four days. The remaining twenty-four rats received SC injections of 0.85% saline

according to the same schedule. Fourteen days after the regimen was completed, behavioral procedures commenced. At this time it was assumed that there would be no remaining behavioral effects of MDMA itself, and there would be significant decreases in 5-HT concentrations as shown in previous reports using identical regimens (Sabol, Lew, Richards, Vosmer, & Seiden, 1996; Scanzello, Hatzidimitriou, Martello, Katz, & Ricuarte, 1993).

Behavioral Procedures

Rats were exposed to dipper training and experimental sessions as described in Experiment 1. Three squads of 8 MDMA-treated rats were each randomly assigned to one delay value of either 0 s, 10 s, or 20 s. Three squads of 8 vehicle-exposed rats (controls) were assigned in the same manner.

Results

All subjects except for two MDMA-treated rats exposed to 20 s delays showed evidence of response acquisition. Data from one MDMA-treated rat exposed to 10-s delay were lost due to equipment failure. Figure 5 shows mean cumulative responses for all groups. Acquisition as evidenced by cumulative responding on the reinforcement lever did not appear to be affected by the MDMA regimen under any of the conditions. Similarly, there was no effect of MDMA treatment on cancellation-lever responding. Individual subject data are shown in the appendix. Both control and MDMA-exposed rats responding under immediate

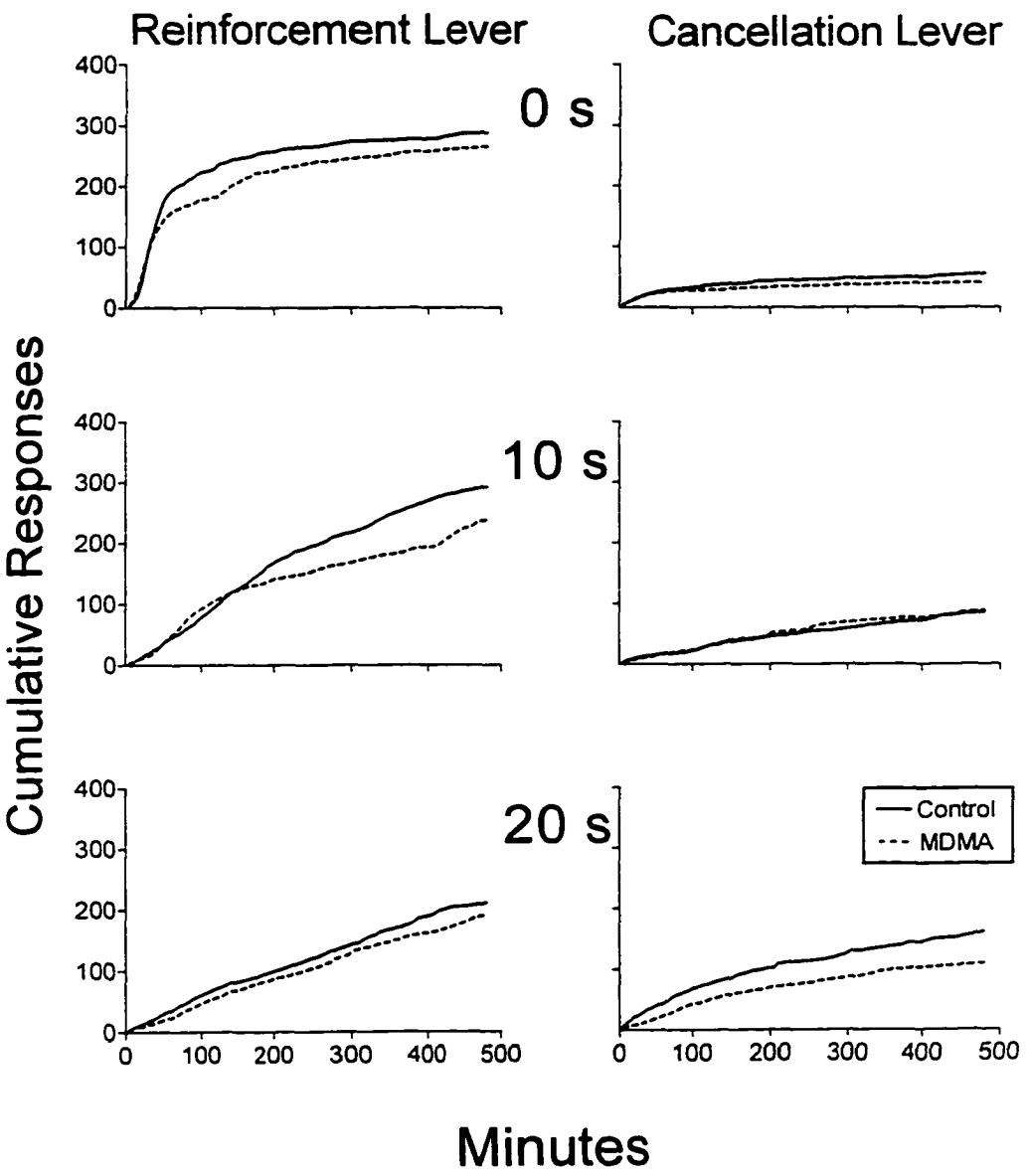


Figure 5. Mean Cumulative Reinforcement-Lever Responses Under Each Experimental Condition.

reinforcement emitted high rates of responding early in the session. Increasing delay decreased lever-pressing for all subjects regardless of MDMA exposure.

Mean total responses and reinforcers earned are shown in Figure 6. There was no effect of drug treatment on total reinforcement-lever responses ($F = .48, p > .05$), and the effect of delay on total reinforcement-lever responses approached statistical significance ($F = 2.83, p = .07$). Differences between mean reinforcers earned by control and MDMA-treated rats approached statistical significance ($F = 3.6, p = .06$) and delay had an inverse relationship to total reinforcers earned ($F = 53.85, p < .01$). There was no interaction of drug and delay on either total responses or reinforcers earned.

Although group data suggest that the MDMA regimen did not affect response acquisition, there are two aspects of the data that may be suggestive of an effect. First and as mentioned above, differences in reinforcers earned between control and MDMA-exposed subjects approached statistical significance. Second, two MDMA-treated rats, 46B and 47B, in the 20-s delay condition did not show evidence of acquisition. These two subjects showed no evidence of accelerated responding and received 4 and 10 reinforcers, respectively, across the 8-hour session. In contrast, the lowest number of reinforcers earned by a control rat exposed to a 20 s reinforcement delay was 40, and that subject exhibited accelerated rates of responding towards the end of the session. However, other MDMA-treated subjects exhibited strong evidence of acquisition. As seen in Figure 7, which shows individual cumulative records from the highest and lowest responders from each group exposed to 20-s

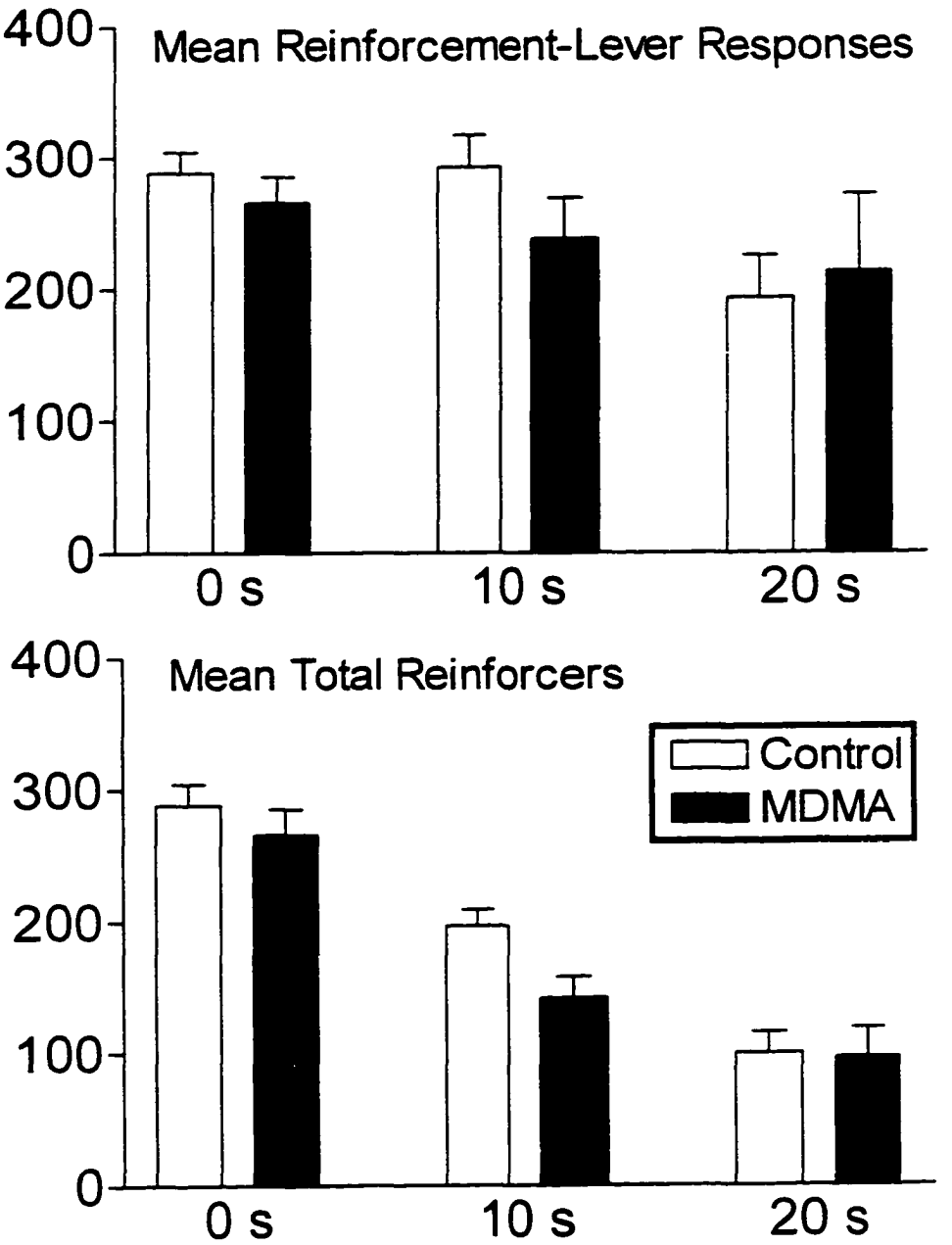


Figure 6. Mean Total Reinforcement-Lever Presses and Total Reinforcers Earned Under Each Experimental Condition.

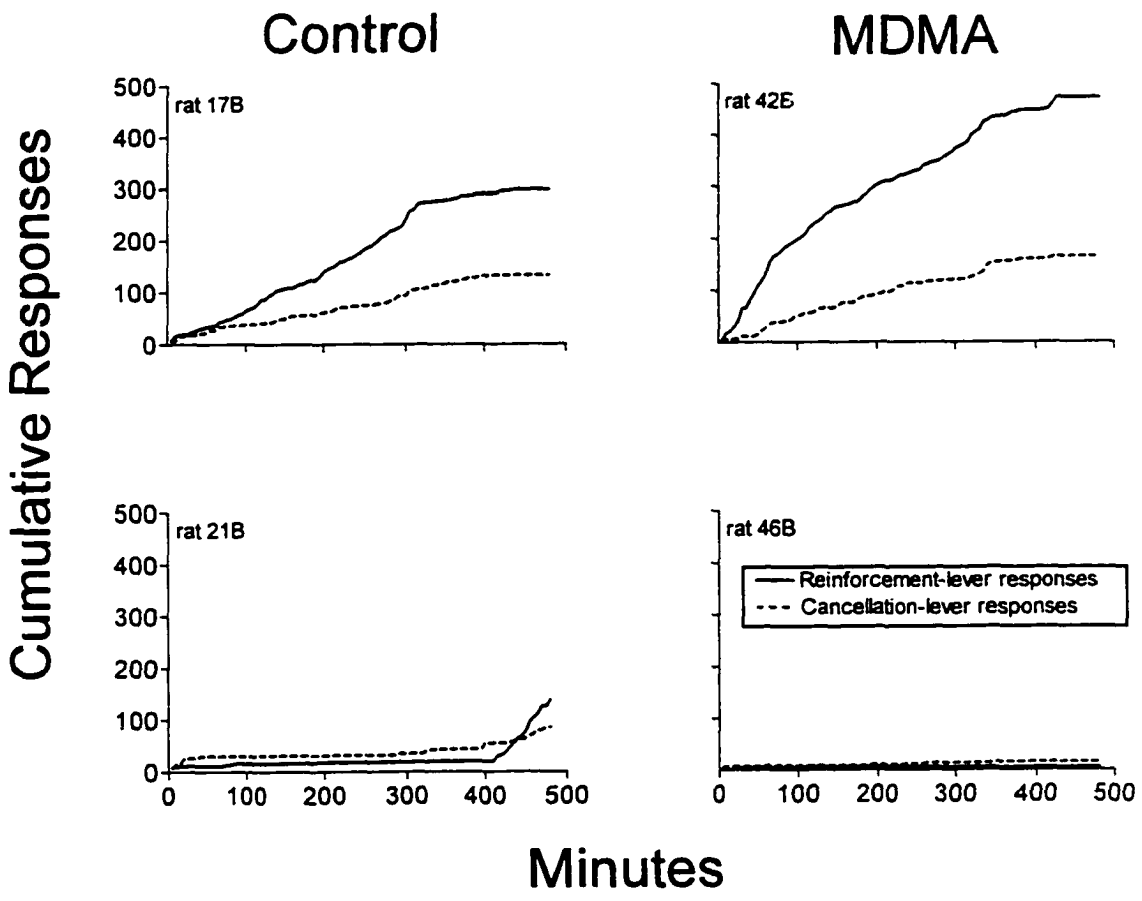


Figure 7. Comparison of Highest and Lowest Responders From Control and MDMA Groups Exposed to 20 s Reinforcement Delays.

reinforcement delays, MDMA-treated rat 42B emitted 473 reinforcement-lever responses and received 176 reinforcers. No control rat matched this performance. High variability across MDMA-treated rats may itself indicate some effect of drug, but there is no convincing evidence that the regimen interfered with learning.

Discussion

The finding that MDMA-induced neurotoxicity did not interfere with learning is consistent with other studies examining the neurotoxic effects of the drug on learning (Frederick et al., 1995a) and memory (LeSage et al., 1993). As stated by Frederick et al., there are many possible reasons why MDMA-induced, 5-HT depletion may not seem to affect behavior. First, the neurotoxic effects may not be large enough to cause any detectable behavioral deficits. For example, Ricuarte et al. (1993) found that serotonergic deficits caused by MDMA did not affect behavior unless combined with serotonin damage caused by another neurotoxin. Other explanations proposed by Frederick are that 5-HT may not play an important role in a given learning task or other brain systems may compensate for any 5-HT deficits. The last possibility is that the specific assay used is not sensitive to the behavioral effects of the neurotoxicity. This last possibility could be examined further by changing delay parameters. Longer delays may make learning more difficult and more susceptible to perturbations. Conducting a replication of the current study with a larger pool of subjects may also indicate whether the failure of two rats to acquire in the current assay was a chance occurrence or an effect of neurotoxicity.

CHAPTER IV

GENERAL DISCUSSION

To my knowledge, this is the first report describing the effects of MDMA on the acquisition of new, operant behavior. Data from these two experiments suggest that acute and neurotoxic regimens of MDMA do not retard response acquisition.

It appears that the acute effects of MDMA on response acquisition are similar to *d*-amphetamine in that low to medium doses of the drug either enhance or have no effect on measures of learning (LeSage et al.,, 1996; Thompson, 1974). Higher doses of *d*-amphetamine seem to interfere with learning only by producing general behavior disruption (Evans & Wenger, 1992); in the current study the highest dose of MDMA (5.6 mg/kg) retarded acquisition only at the beginning of the session which is a similar effect found under similar acquisition procedures with *d*-amphetamine. It is also notable that neither drug seemed to affect proportion of responding emitted on the lever that did not produce reinforcement. Hence, the effects of MDMA and *d*-amphetamine seem to be similar under response acquisition assays which is consistent with research showing that both drugs may produce similar effects on schedule-controlled behavior (LeSage & Poling, 1997).

Currently, there is substantial scientific and social interest in MDMA, and such interest can be attributed, in large part, to the drug's unique discriminative

properties as well as its neurotoxic capacity. At this time, there is no strong evidence that the neurological perturbations caused by MDMA administration have any detrimental behavioral correlates. Although there is a possibility that yet undiscovered behavioral deficits may arise from chronic MDMA self-administration, the danger does not appear to be great.

This study supports a growing body of research that demonstrates that naive organisms can acquire operant behavior when reinforcement is delayed in the absence of any direct shaping. Although longer delays seem to produce variability among subjects exposed to the same conditions, acquisition under these procedures is robust. The present findings, along with those reported by LeSage et al. (1996) and Byrne et al. (1997) suggest that these assays are capable of providing clear measures of drug effects on new, operant behavior.

This is the first study to report drug effects on acquisition using a resetting/cancellation procedure. This is also the first study to use such procedures in an attempt to detect toxic drug effects. Because such effects were not found with MDMA, the utility of these procedures for toxicology remains to be seen. It would seem worthwhile to test toxins with known effect on learning to evaluate the assay's potential.

Appendix A

Individual Subject Data for Experiment 1

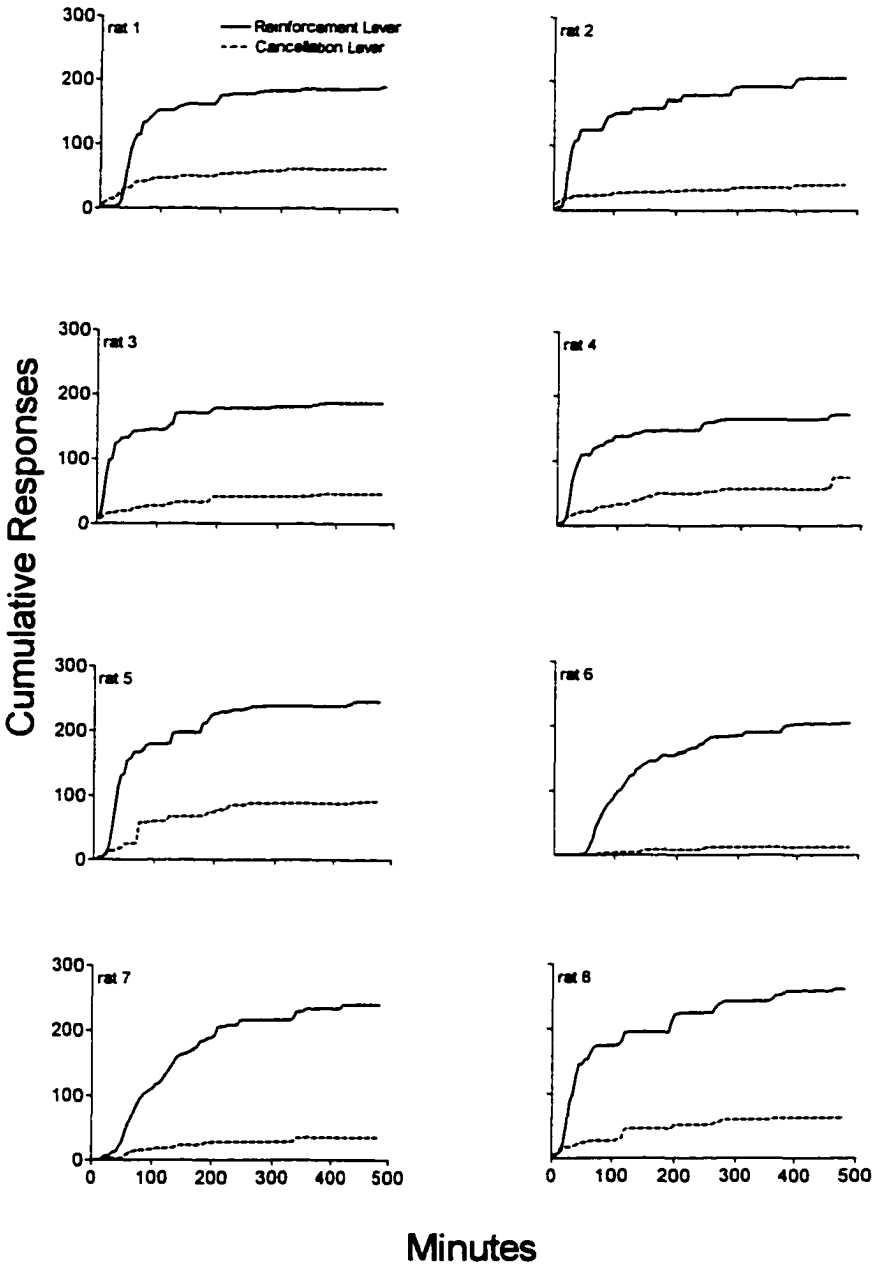


Figure 8. Cumulative Responses on the Reinforcement and Cancellation Levers by Individual Rats Exposed to Immediate Reinforcement and Vehicle.

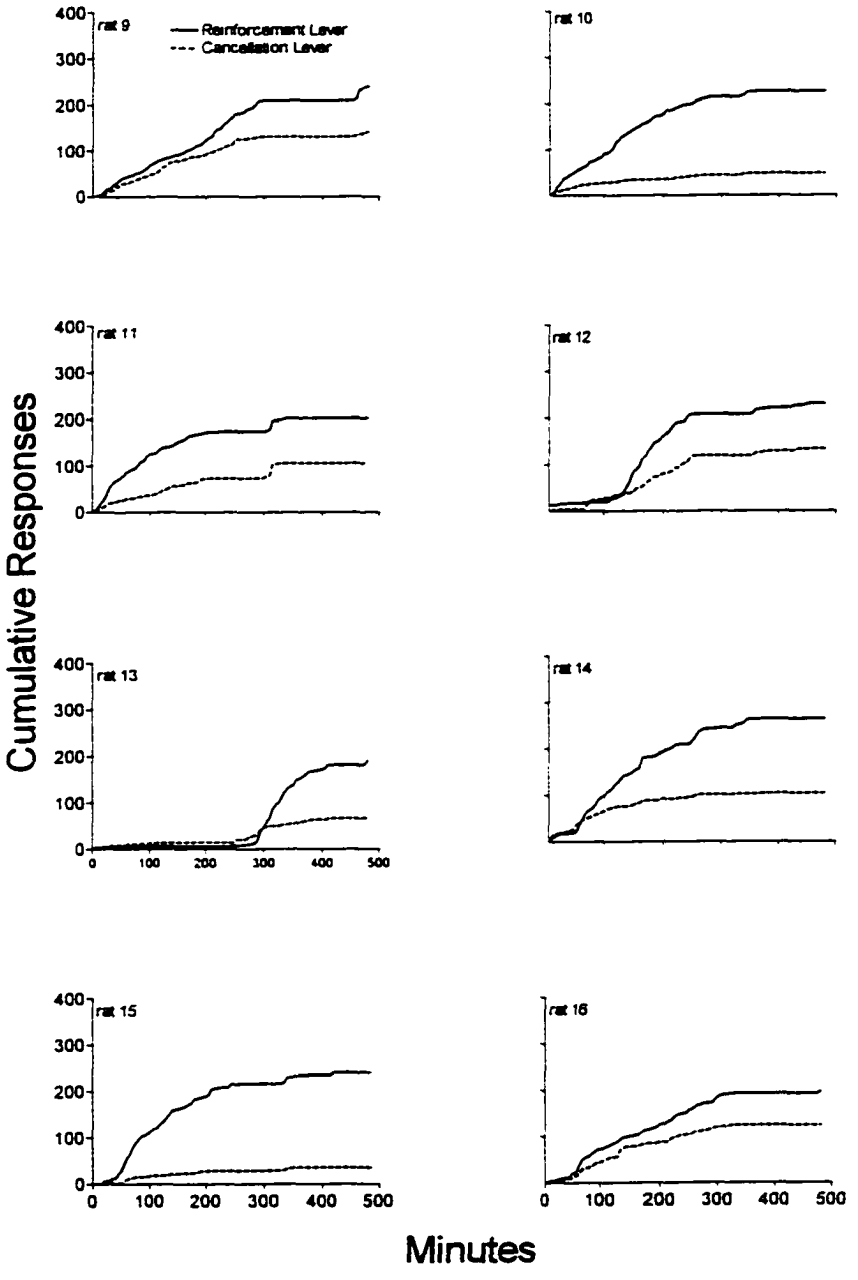


Figure 9. Cumulative Responses on the Reinforcement and Cancellation Levers by Individual Rats Exposed to a 10-s Reinforcement Delay and Vehicle.

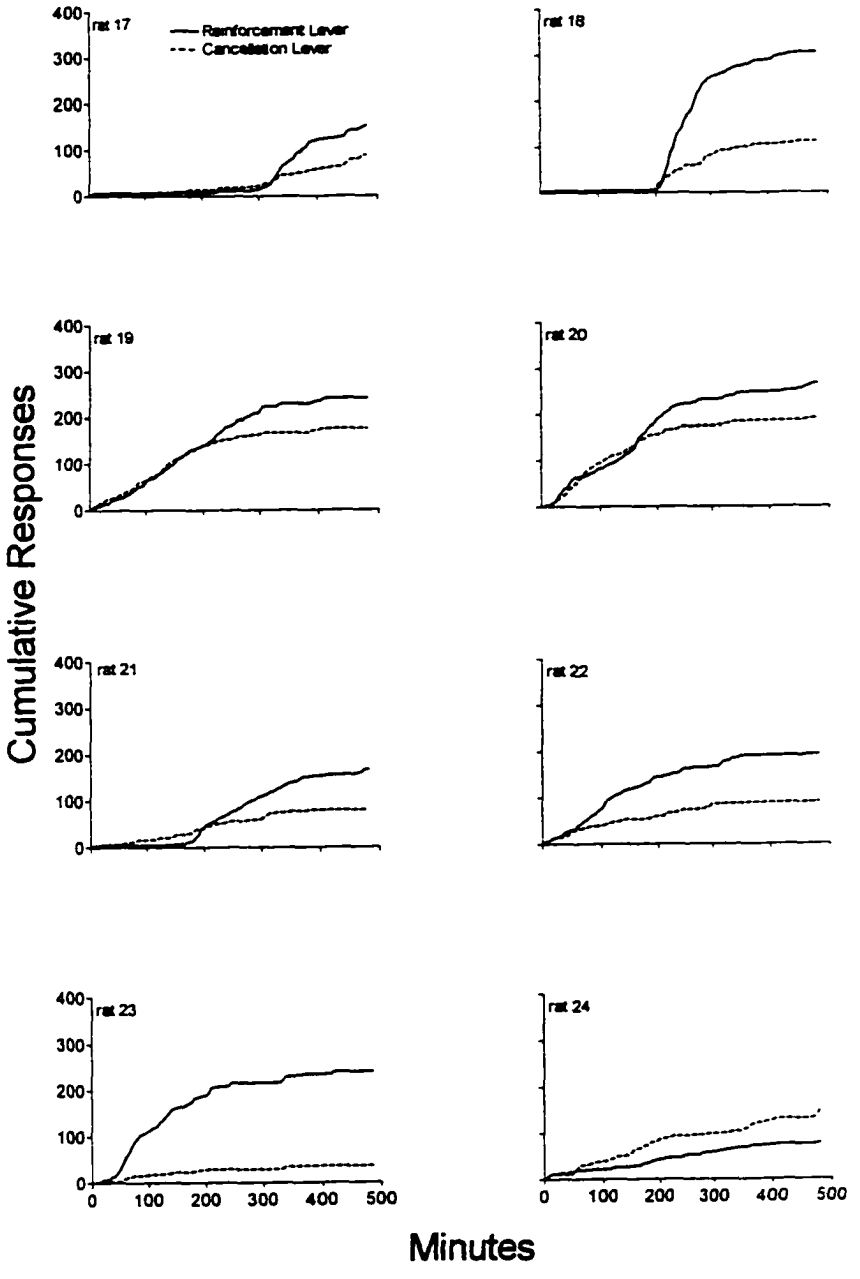


Figure 10. Cumulative Responses on the Reinforcement and Cancellation Levers by Individual Rats Exposed to a 20-s Reinforcement Delay and Vehicle.

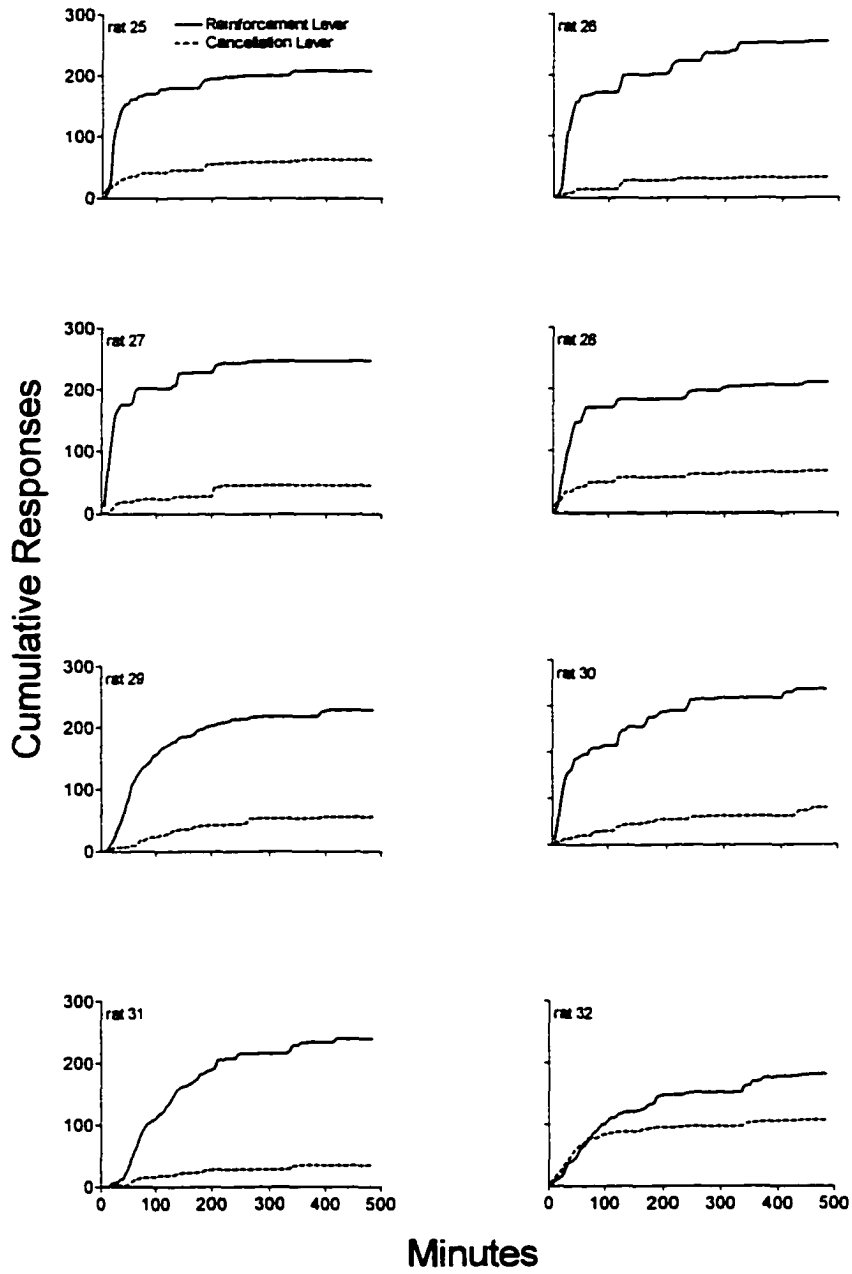


Figure 11. Cumulative Responses on the Reinforcement and Cancellation Levers by Individual Rats Exposed to Immediate Reinforcement and 1.0 mg/kg MDMA.

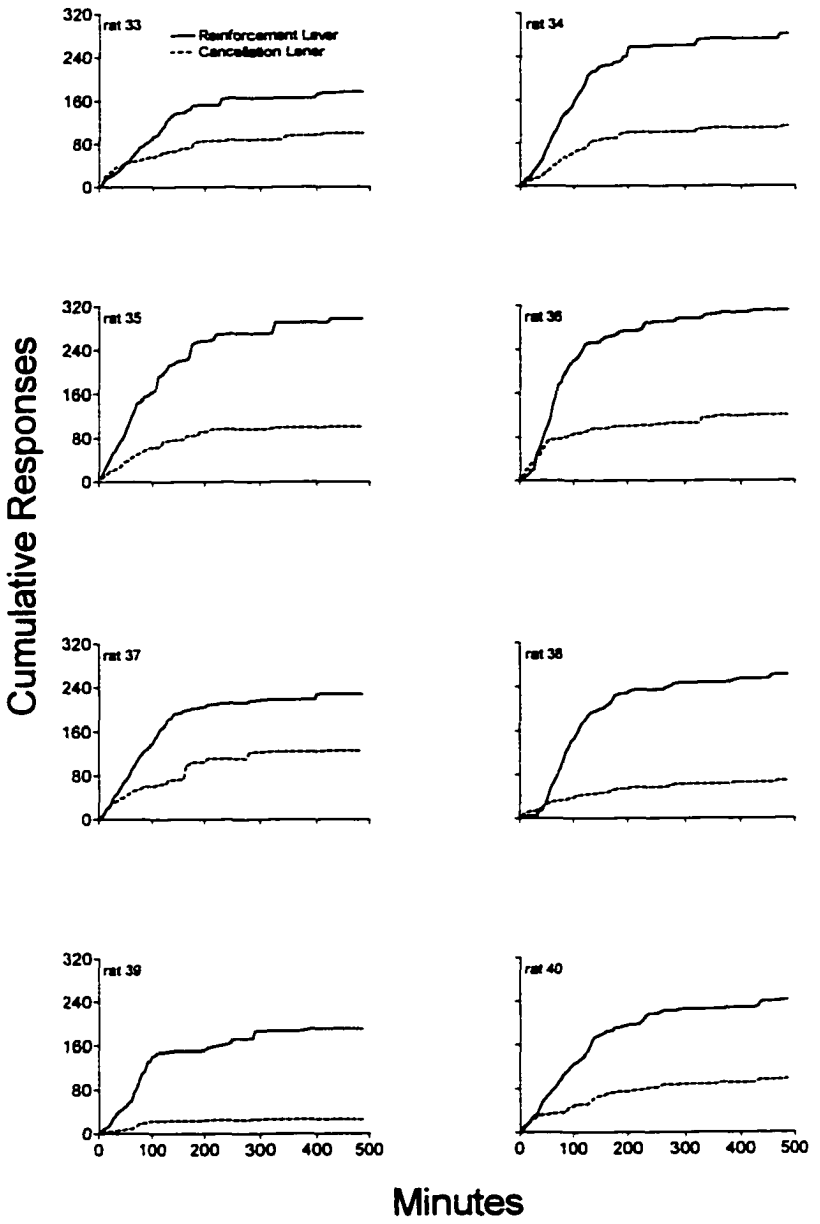


Figure 12. Cumulative Responses on the Reinforcement and Cancellation Levers by Individual Rats Exposed to a 10-s Reinforcement Delay and 1.0 mg/kg MDMA.

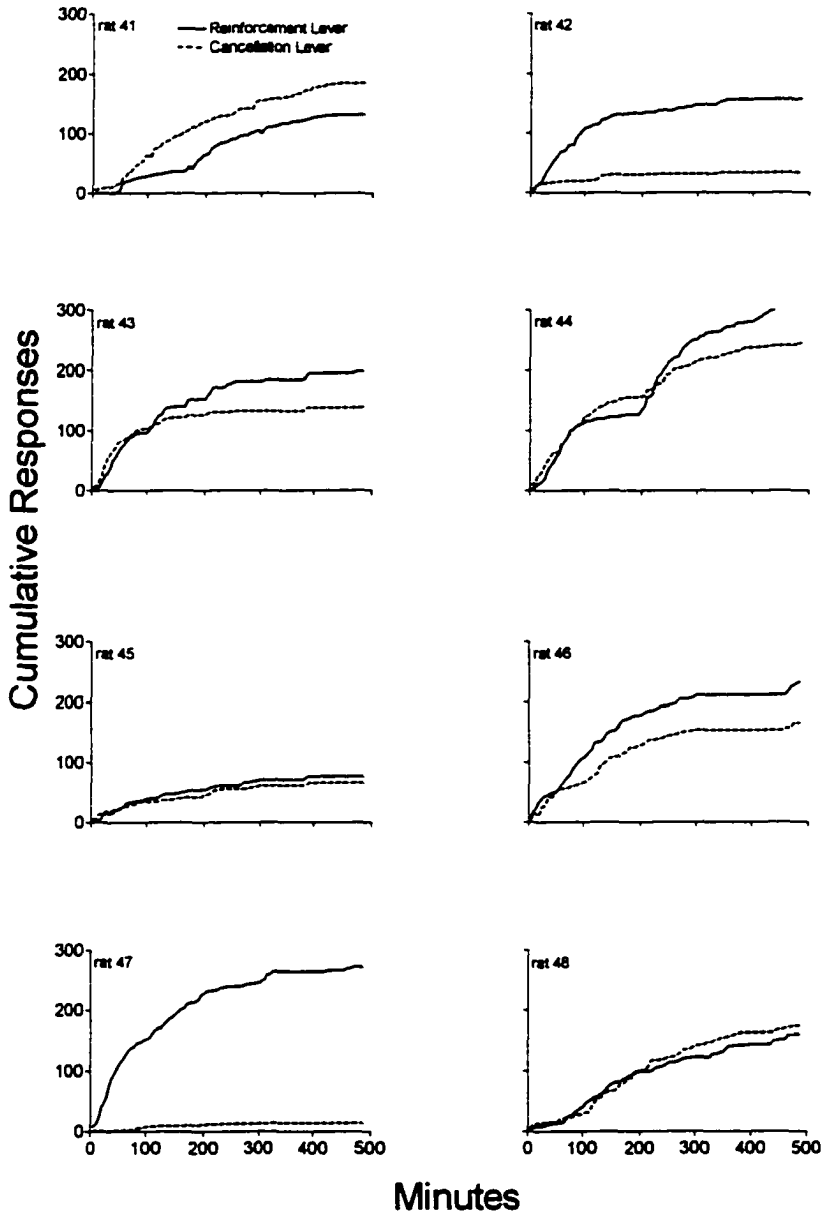


Figure 13. Cumulative Responses on the Reinforcement and Cancellation Levers by Individual Rats Exposed to a 20-s Reinforcement Delay and 1.0 mg/kg MDMA.

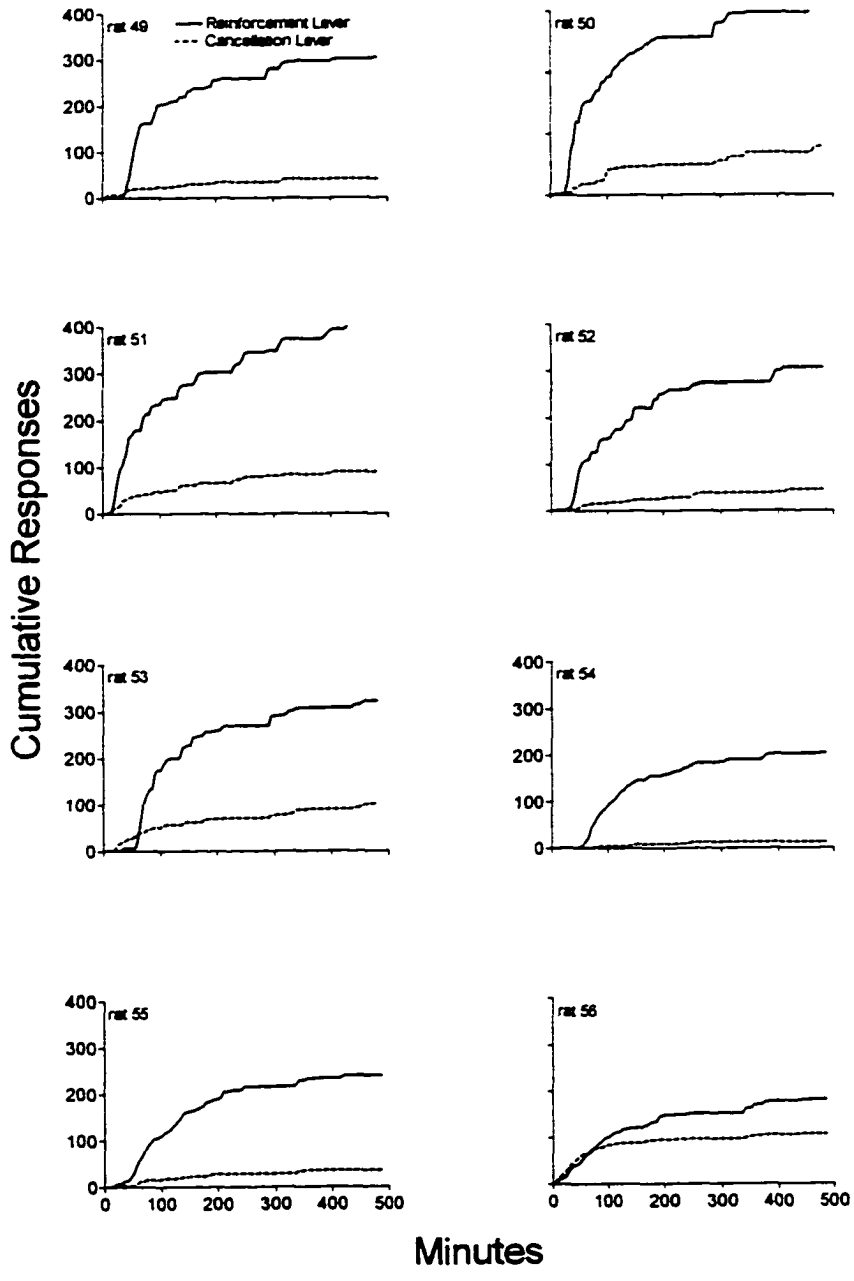


Figure 14. Cumulative Responses on the Reinforcement and Cancellation Levers by Individual Rats Exposed to Immediate Reinforcement and 3.2 mg/kg MDMA.

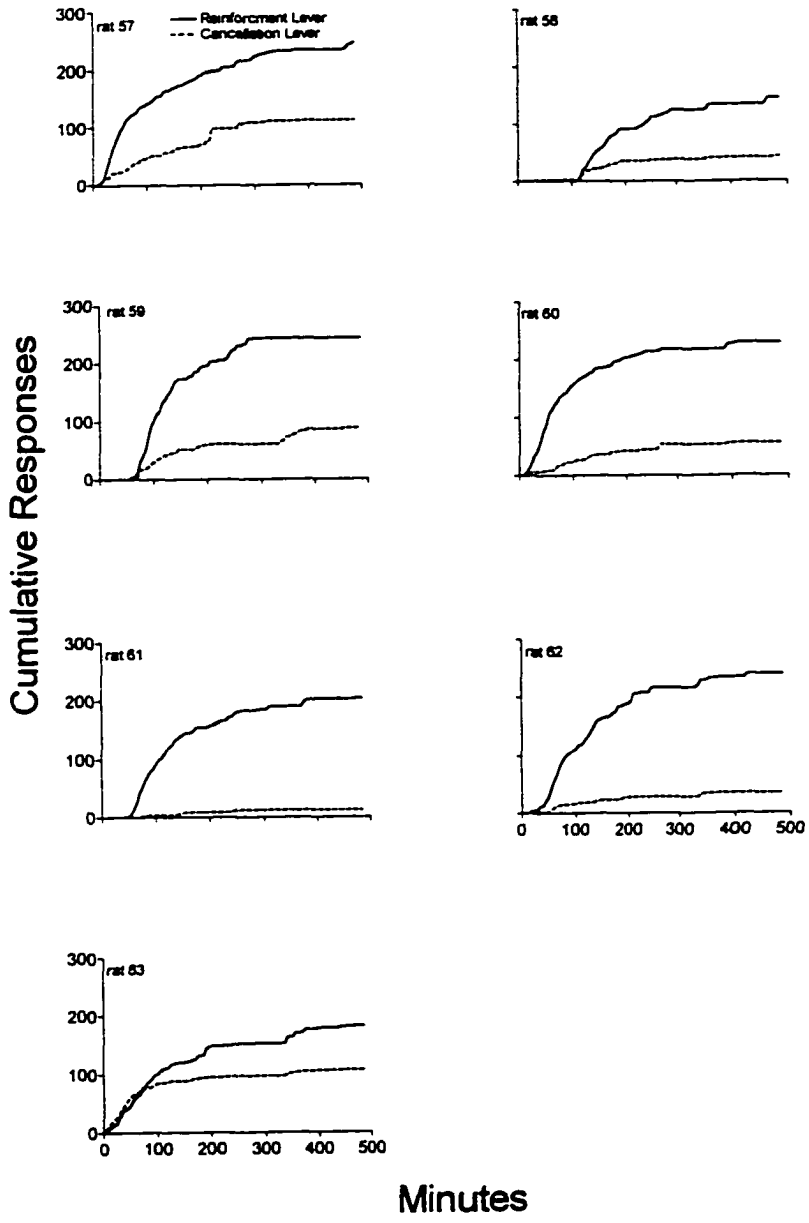


Figure 15. Cumulative Responses on the Reinforcement and Cancellation Levers by Individual Rats Exposed to a 10-s Reinforcement Delay and 3.2 mg/kg MDMA.

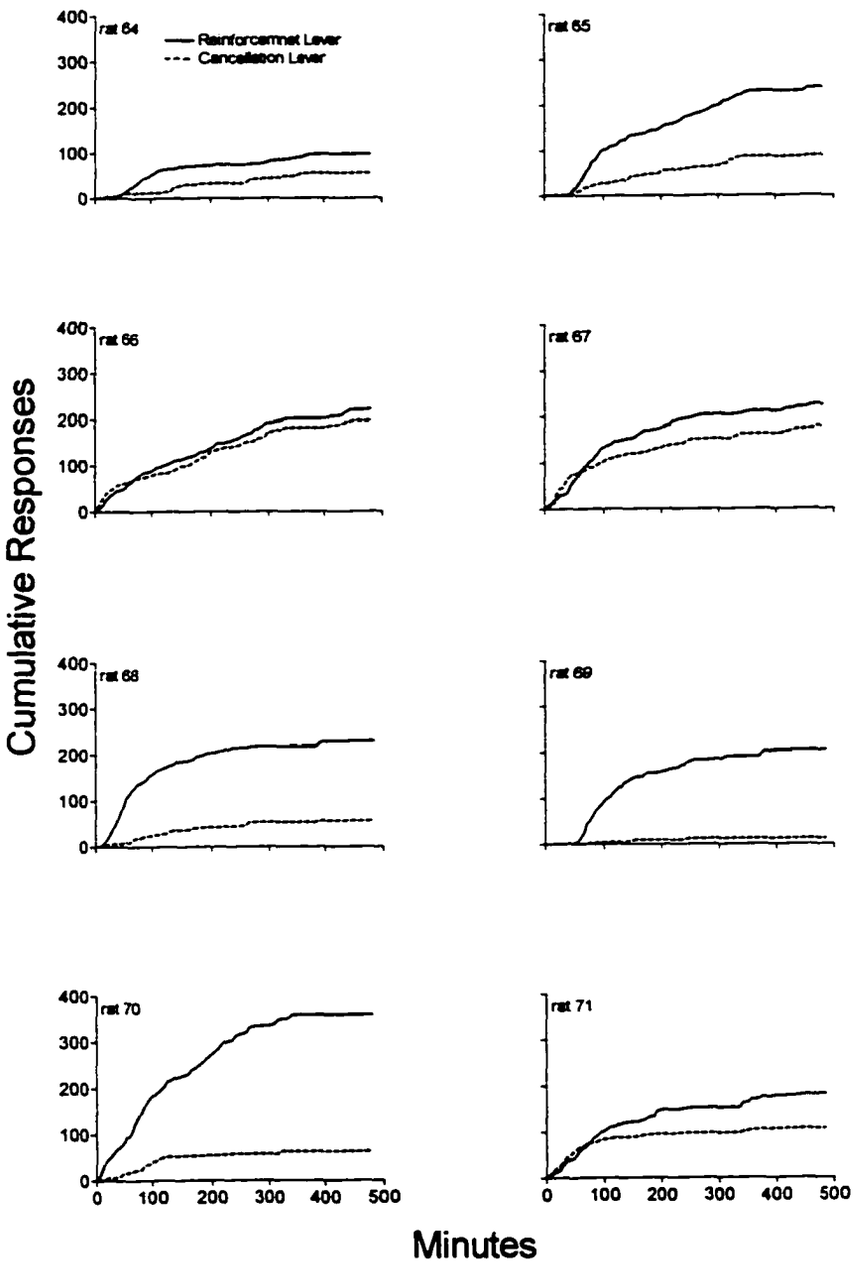


Figure 16. Cumulative Responses on the Reinforcement and Cancellation Levers by Individual Rats Exposed to a 20-s Reinforcement Delay and 3.2 mg/kg MDMA.

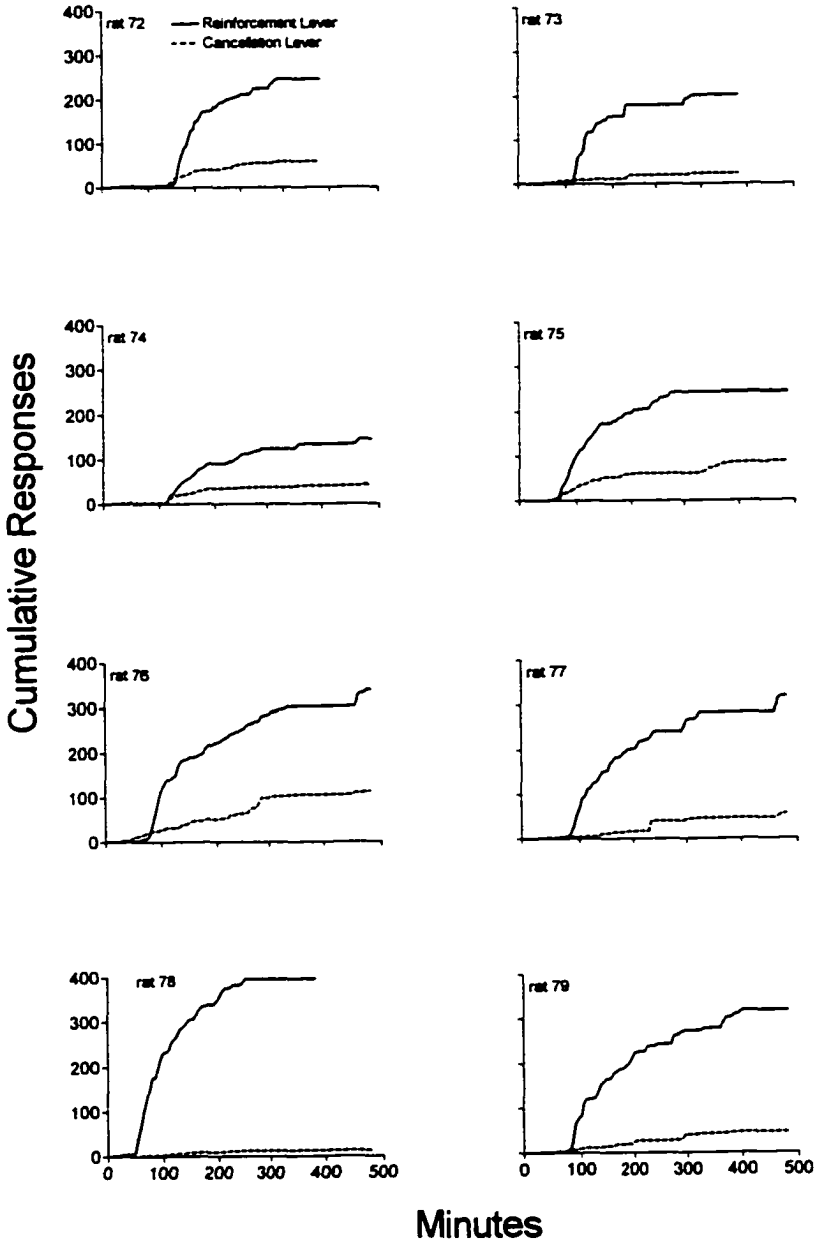


Figure 17. Cumulative Responses on the Reinforcement and Cancellation Levers by Individual Rats Exposed to Immediate Reinforcement and 5.6 mg/kg MDMA.

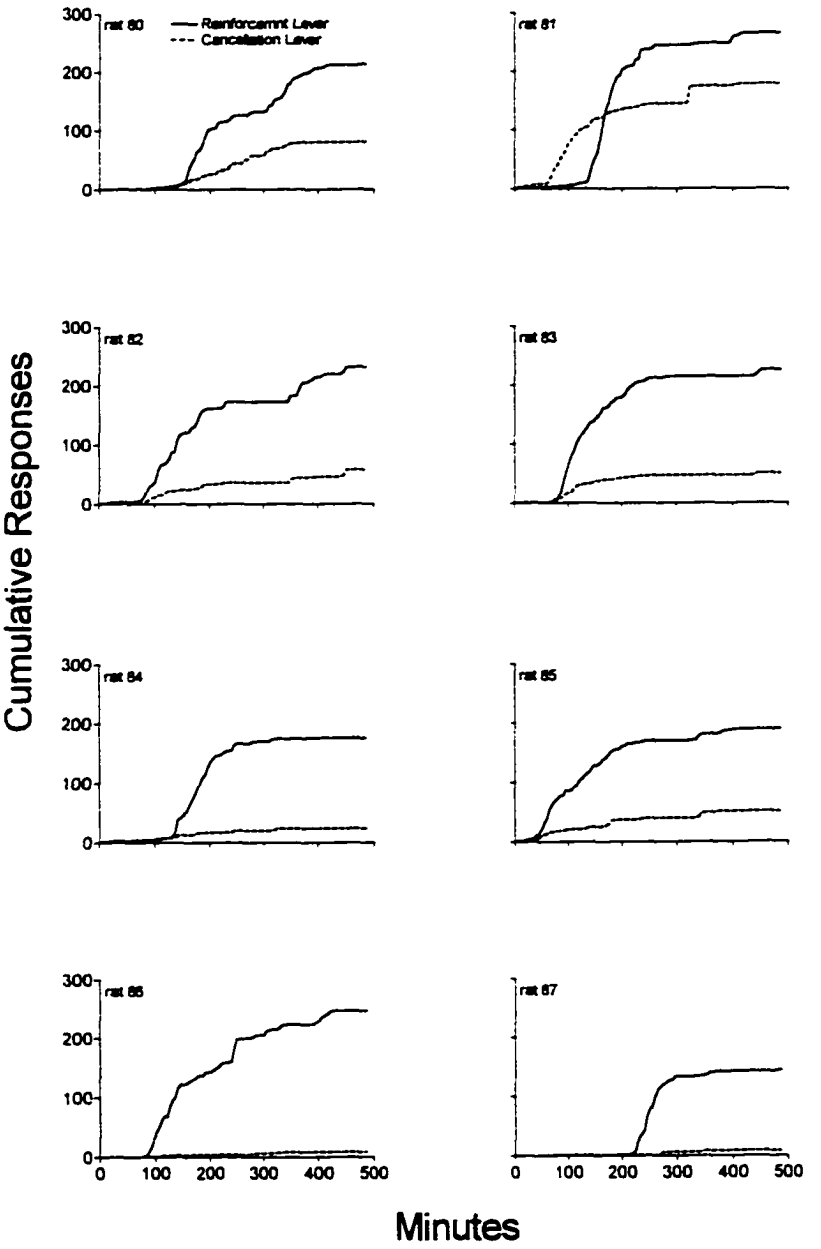


Figure 18. Cumulative Responses on the Reinforcement and Cancellation Levers by Individual Rats Exposed to a 10-s Reinforcement Delay and 5.6 mg/kg MDMA.

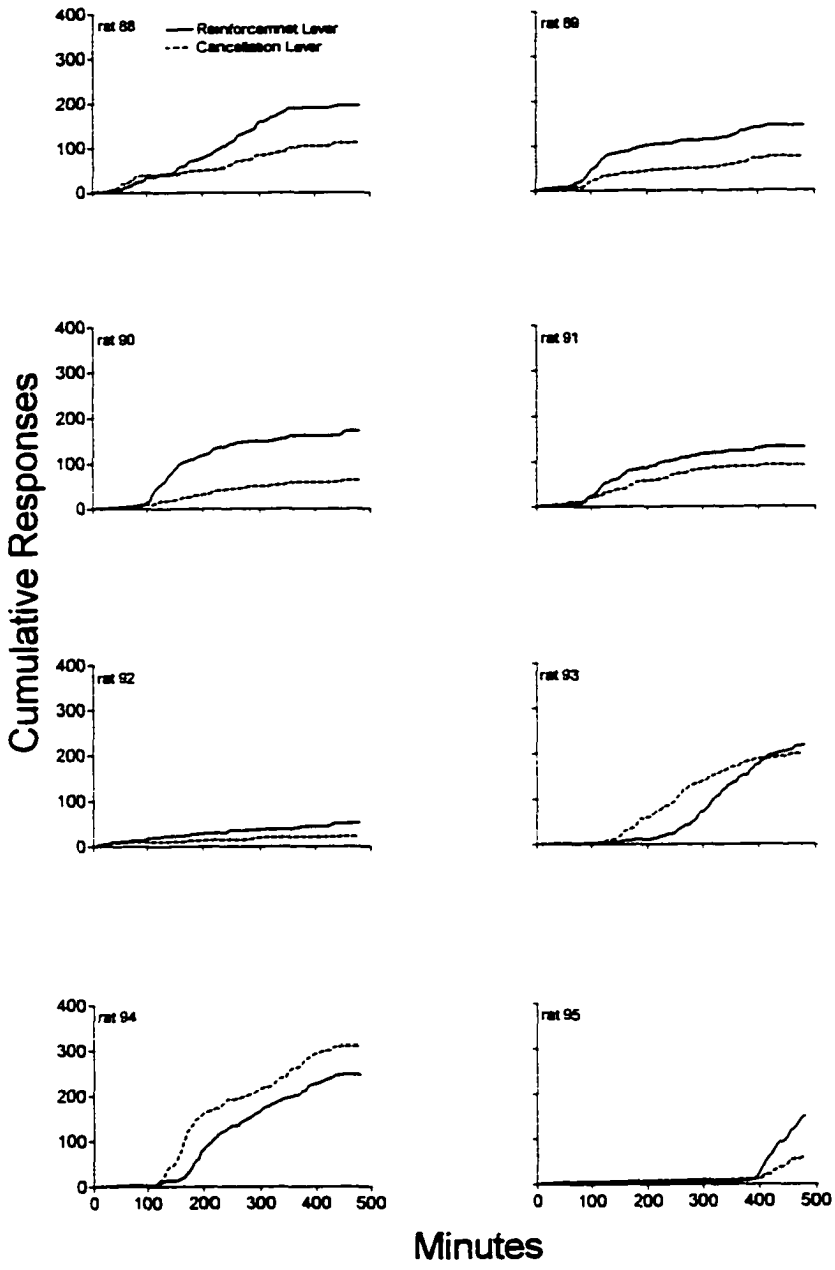


Figure 19. Cumulative Responses on the Reinforcement and Cancellation Levers by Individual Rats Exposed to a 20-s Reinforcement Delay and 5.6 mg/kg MDMA.

Table 1

Water Deliveries, Lever-presses, and Latency Until First 50 Lever Presses for all Subjects in Experiment 1.

Condition	Subject	Water deliveries	Operative-lever response	Cancellation-lever responses	Minutes until 50 lever presses
Vehicle 0 s	1	189	189	62	40
	2	206	206	40	25
	3	186	186	46	15
	4	171	171	75	20
	5	245	245	91	30
	6	151	251	55	20
	7	203	203	31	10
	8	262	262	63	25
	Group *M*	201.6	201.6	57.9	23.1
Vehicle 10 s	9	150	239	141	40
	10	169	225	47	30
	11	129	201	106	25
	12	127	229	134	105
	13	112	191	66	290
	14	173	263	104	50
	15	144	240	34	40
	16	136	198	124	65
	Group *M*	114.0	223.3	94.5	80.63
Vehicle 20 s	17	68	153	88	320
	18	86	303	111	210
	19	105	241	176	90
	20	117	266	190	50
	21	88	168	82	185
	22	112	194	91	50
	23	98	287	36	110
	24	44	78	148	80
	Group *M*	89.7	211.3	115.3	136.9
1.0 mg/kg 0 s	25	208	208	63	20
	26	255	255	33	20
	27	247	247	45	10
	28	210	210	67	15
	29	341	341	87	15
	30	336	336	79	15
	31	282	282	27	15
	32	214	214	93	15
	Group *M*	261.6	261.6	61.75	15.6
1.0 mg/kg 10 s	33	126	177	100	25
	34	170	281	111	30
	35	154	298	101	20
	36	169	311	121	20
	37	131	228	126	25

Table 1—Continued

Condition	Subject	Water deliveries	Operative-lever responses	Cancellation-lever responses	Minutes until 50 lever presses
	37	131	228	126	25
	38	149	262	69	35
	39	105	191	26	45
	40	152	242	99	25
	Group *M*	144.5	248.8	94.1	22.5
1.0 mg/kg 20 s	41	73	131	184	65
	42	83	157	33	30
	43	80	198	139	25
	44	111	318	244	30
	45	40	76	66	60
	46	113	231	165	25
	47	141	272	14	30
	48	89	159	173	85
	Group *M*	91.3	192.8	127.3	43.75
3.2 mg/kg 0 s	49	305	305	41	45
	50	311	311	79	35
	51	411	411	90	30
	52	305	305	45	45
	53	323	323	101	60
	54	371	371	53	30
	55	266	266	19	45
	56	282	282	61	50
	Group *M*	321.8	321.8	61.1	42.5
3.2 mg/kg 10 s	57	152	248	113	30
	58	106	145	43	130
	59	118	243	88	80
	60	142	229	56	40
	61	135	204	12	75
	62	132	240	35	55
	63	113	182	107	30
	Group *M*	109.9	213	64.9	62.9
3.2 mg/kg 20 s	64	60	97	55	90
	65	96	236	88	65
	66	112	221	196	15
	67	96	225	177	20
	68	22	39	100	90
	69	118	268	147	25
	70	145	359	63	25
	71	73	160	282	15
	Group *M*	90.25	200.6	138.5	43.1

Table 1—Continued

Condition	Subject	Water deliveries	Operative-lever response	Cancellation-lever responses	Minutes until 50 lever presses
5.6 mg/kg 0 s	72	247	247	59	165
	73	202	202	23	125
	74	315	315	56	100
	75	389	389	75	25
	76	340	340	114	85
	77	318	318	56	95
	78	342	342	12	60
	79	318	318	46	90
	Group *M*	308.9	308.9	55.1	93.1
5.6 mg/kg 10 s	80	115	214	81	165
	81	128	265	178	85
	82	116	232	58	105
	83	123	225	50	95
	84	110	176	24	140
	85	126	190	51	60
	86	138	247	8	105
	87	75	144	8	245
	Group *M*	116.4	211.6	57.25	125
5.6 mg/kg 20 s	88	115	195	110	85
	89	82	145	75	95
	90	73	172	64	115
	91	53	132	92	110
	92	13	22	52	275
	93	97	217	199	165
	94	103	248	316	135
	95	64	150	59	405
	Group *M*	75	160.1	120.9	173.1

Appendix B

Individual Subject Data for Experiment 2

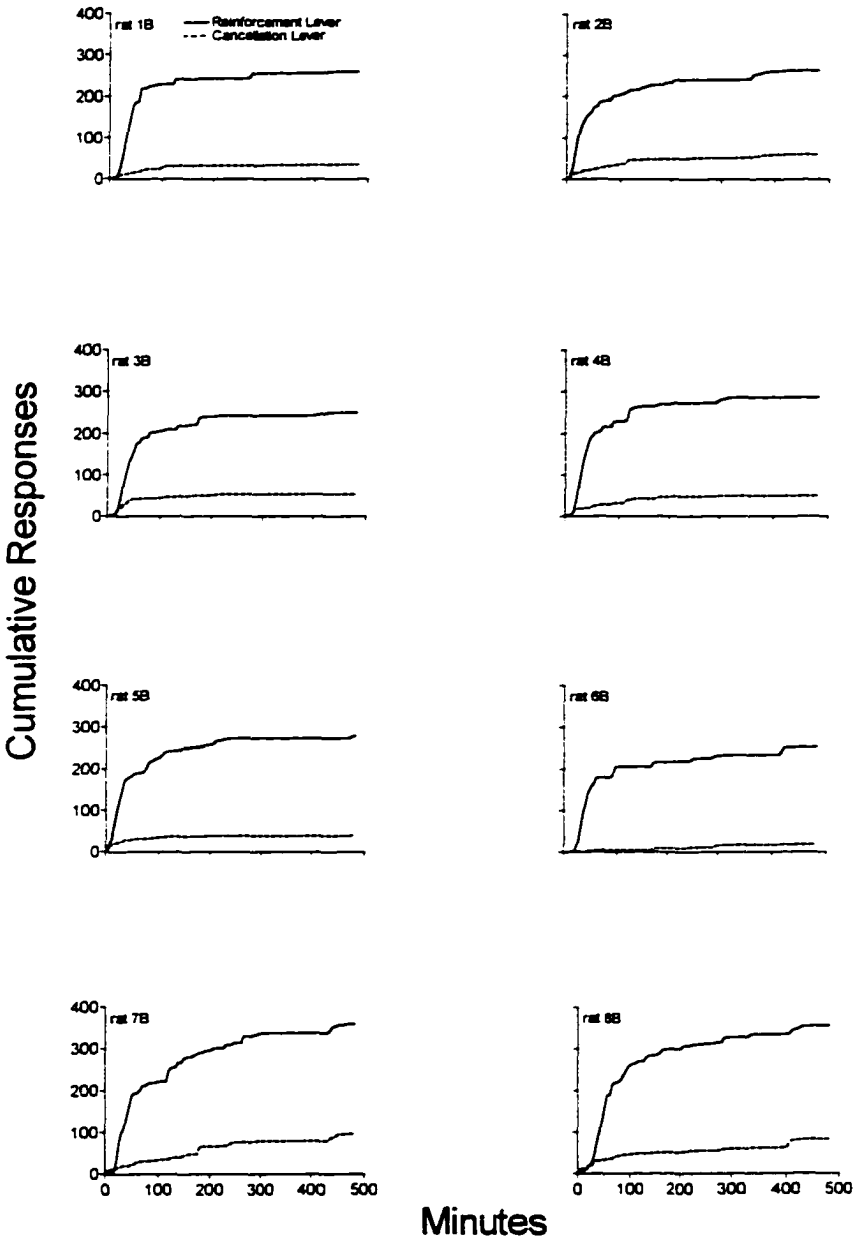


Figure 20. Cumulative Responses on the Reinforcement and Cancellation Levers by Vehicle-Control Rats in the Immediate Reinforcement Condition.

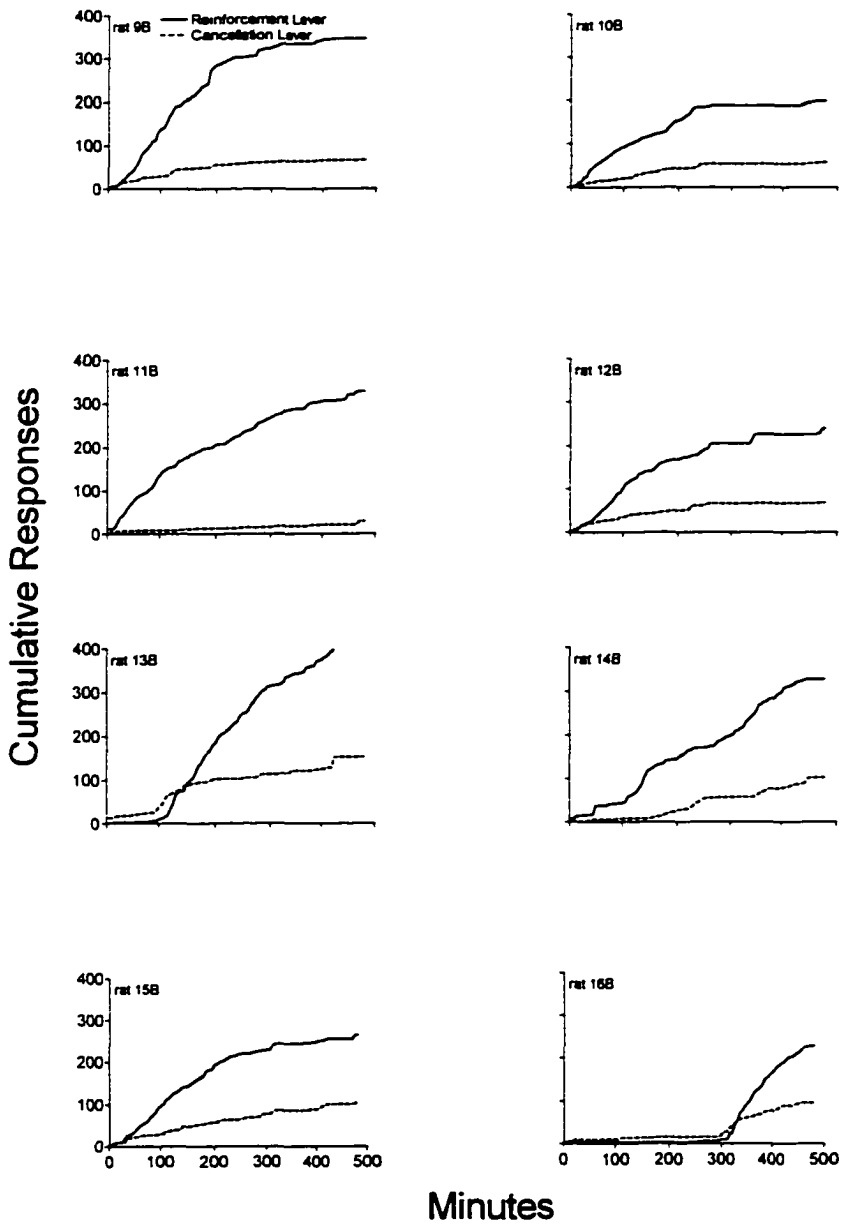


Figure 21. Cumulative Responses on the Reinforcement and Cancellation Levers by Vehicle-Exposed Rats in the 10-s Delay Condition.

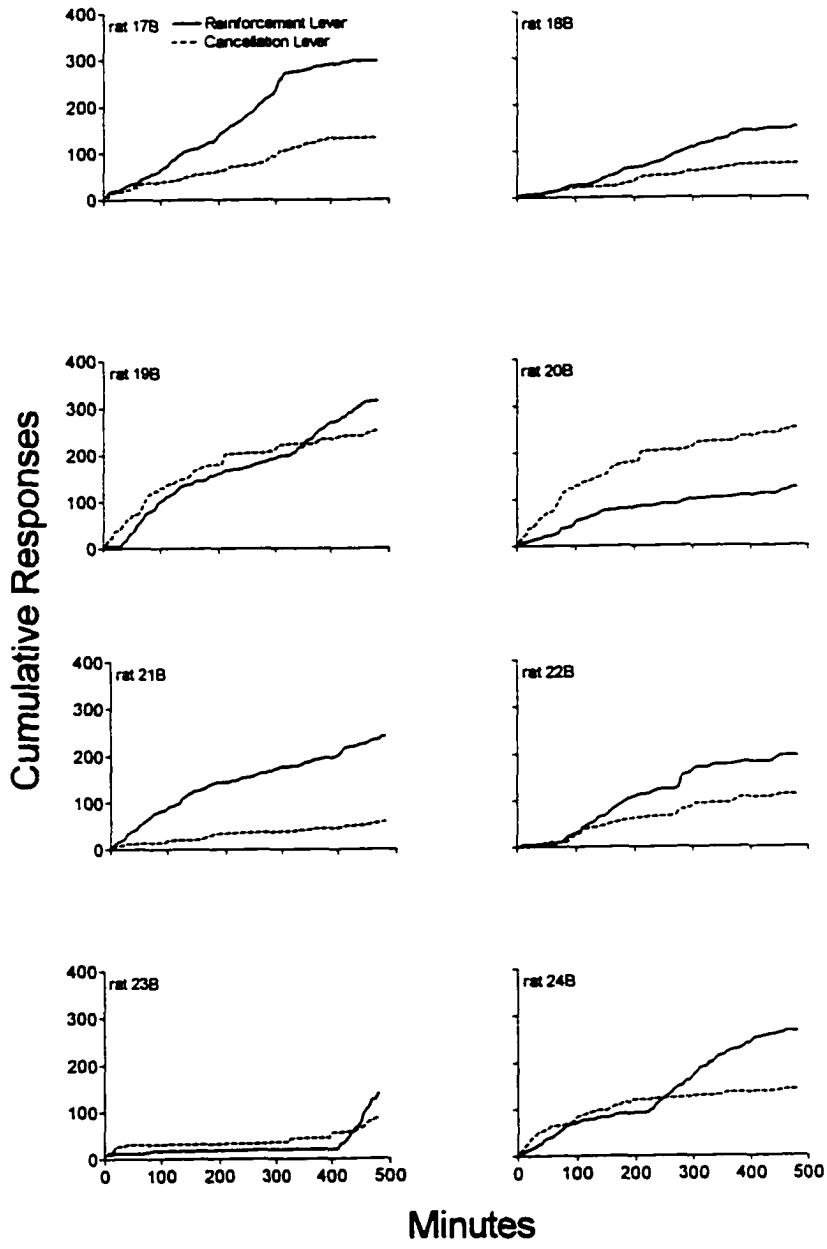


Figure 22. Cumulative Responses on the Reinforcement and Cancellation Levers by Vehicle-Exposed Rats in the 20-s Delay Condition.

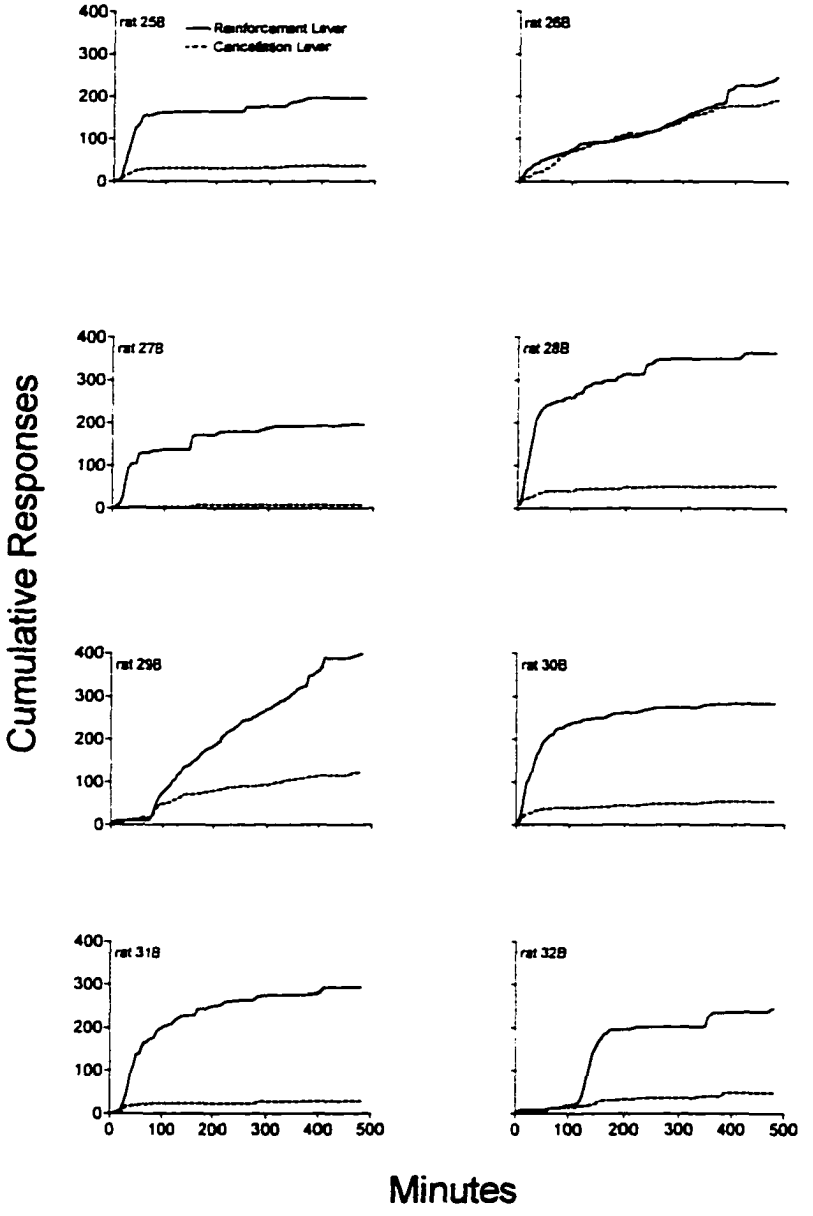


Figure 23. Cumulative Responses on the Reinforcement and Cancellation Levers by MDMA-Exposed Rats in Immediate Reinforcement Condition.

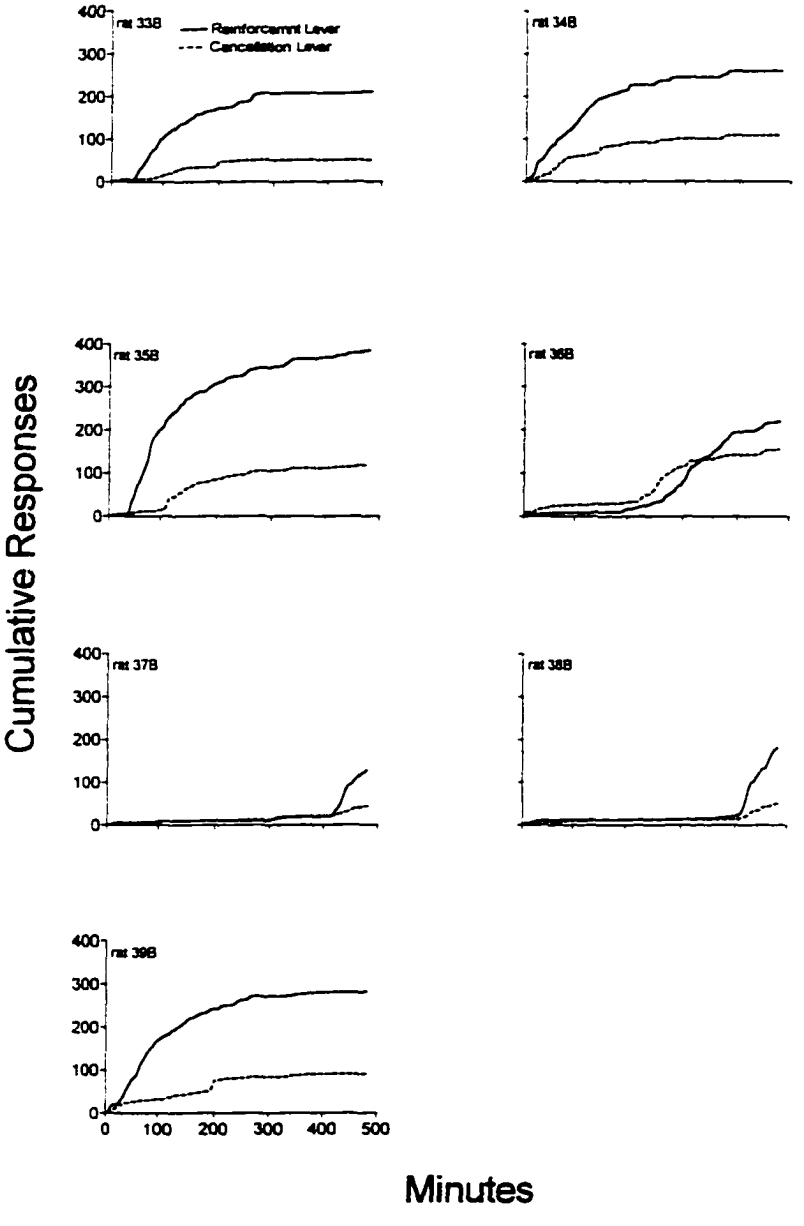


Figure 24. Cumulative Responses on the Reinforcement and Cancellation Levers by MDMA-Exposed Rats in the 10-s Delay Condition.

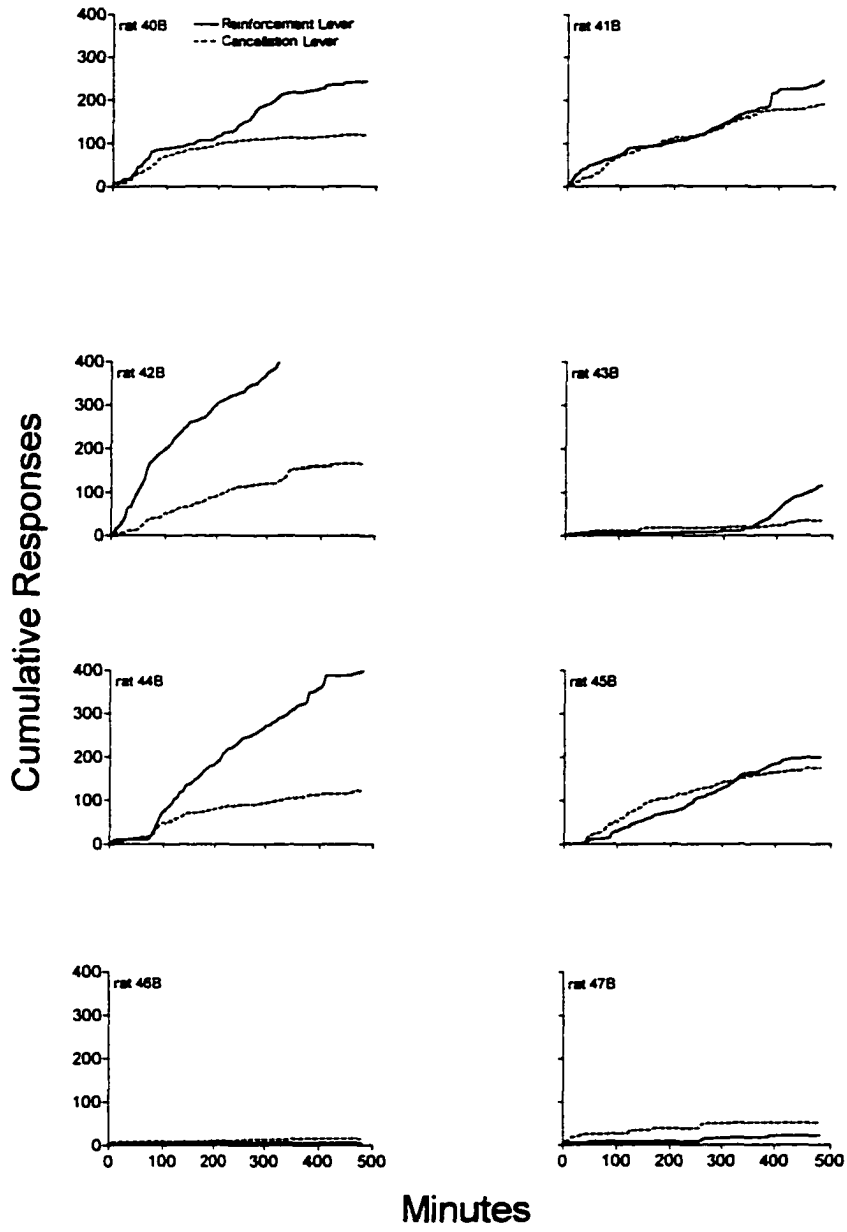


Figure 25. Cumulative Responses on the Reinforcement and Cancellation Levers by MDMA-Exposed Rats in the 20-s Delay Condition.

Table 2

Water deliveries, lever-presses, and latency until first 50 lever presses for all subjects in Experiment 2.

Condition	Subject	Water deliveries	Operative-lever response	Cancellation-lever responses	Minutes until 50 lever presses
Control 0 s	1B	260	260	36	25
	2B	263	263	61	15
	3B	250	250	54	25
	4B	286	286	50	20
	5B	279	279	42	15
	6B	252	252	18	30
	7B	360	360	97	25
	8B	356	356	83	30
	Group *M*	288.3	288.3	55.1	23.1
Control 10 s	9B	347	242	66	40
	10B	198	172	58	35
	11B	328	217	29	30
	12B	240	203	69	45
	13B	405	244	154	110
	14B	328	175	103	90
	15B	265	174	106	45
	16B	228	150	96	315
	Group *M*	292.4	197.1	85.1	88.8
Control 20 s	17B	298	147	133	40
	18B	151	108	72	125
	19B	314	150	252	35
	20B	124	77	252	30
	21B	59	36	242	40
	22B	195	109	112	100
	23B	139	40	210	86
	24B	265	132	142	25
	Group *M*	193.1	99.9	176.9	60.1
MDMA 0 s	25B	196	196	36	25
	26B	257	257	49	15
	27B	196	196	7	25
	28B	364	364	52	10
	29B	293	293	52	20
	30B	283	283	54	15
	31B	292	292	29	30
	32B	244	244	48	125
	Group *M*	265.6	265.6	40.9	33.1

Table 2—Continued

Condition	Subject	Water deliveries	Operative-lever response	Cancellation-lever responses	Minutes until 50 lever presses
MDMA 10 s	33B	212	159	51	65
	34B	260	162	109	20
	35B	384	175	118	50
	36B	219	155	154	195
	37B	129	63	44	420
	38B	181	99	50	415
	39B	282	179	91	35
	Group *M*	238.1	141.7	88.1	171.4
MDMA 20 s	40B	244	110	120	40
	41B	247	127	191	25
	42B	473	176	165	30
	43B	116	65	34	95
	44B	399	159	123	80
	45B	199	120	174	80
	46B	5	4	15	NA
	47B	22	10	52	260
	Group *M*	213.1	96.4	109.3	87.1

Appendix C

IACUC Approval

WESTERN MICHIGAN UNIVERSITY
INVESTIGATOR IACUC CERTIFICATE

Title of Project: Acute and chronic effects of MDMA on response acquisition with delayed reinforcement

The information included in this IACUC application is accurate to the best of my knowledge. All personnel listed recognize their responsibility for complying with the university policies regarding 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 or variations form the animal care and use data will be promptly forwarded to the IACUC for approval

____ Disapproved ____ Approved _✓_ Approved with the provisions listed below

Provisions or Explanations.

[illegible handwriting]

[illegible handwriting]

[signature] 2-27-97

IACUC Chairperson Date

Acceptance of Provisions

①

Signature: Principal Investigator/Instructor Date

①

IACUC Chairperson Final Approval Date

Approved IACUC Number 97-02-01

① See signatures on attached pages 3/6/97

BIBLIOGRAPHY

Ali, S. F., Newport, G. D., Scallet, A. C., Binienda, Z., Ferguson, S. A., Bailey, J. R., Paule, M. G., & Slikker, W. (1993). Oral administration of 3,4-methylenedioxmethamphetamine (MDMA) produces selective serotonergic depletions in the nonhuman primate. *Neurotoxicology and Teratology, 15*. 91-96.

Ali, S. F., Scallet, A. C., Holson, R. R., Newport, G. D., & Slikker., W. (1987). Acute administration of MDMA (Ecstasy): Neurochemical changes persist up to 120 days in the rat brain. *Society of Neruoscience Abstracts, 13*, 904.

Alrazi, J., & Vereby, K. (1988). MDMA and biological disposition in Man: MDA is a biotransformation product. In Louis S. Harris (Ed.) *Proceedings of the 50th Annual Scientific Meeting, The Committee on Problems of Drug Dependence, Inc.* (p. 34). Rockville, Maryland: National Institute on Drug Abuse.

Baker, L. E., & Taylor, M. M. (1997). Assesment of the MDA and MDMA optical isomers in a stimulant-hallucinogen paradigm. *Pharmacology Biochemistry and Behavior, 97*, 737-748.

Beardsly, P. M., Balster, R. L., & Harris, L. S. (1986). Self-administration of methylenedioxymethamphetamine (MDMA) by rhesus monkeys. *Drug and Alcohol Dependence, 18*, 149-157.

Branch, M. N. (1977). On the role of "memory" in the analysis of behavior. *Journal of the Experimental Analysis of Behavior, 28*, 171-179.

Branch, M. N. (1991). Behavioral pharmacology. In I. H. Iversen & K. A. Lattal (Eds.), *Experimental analysis of behavior: Part 2* (Vol. 6, pp. 21-77). New York: Elsevier.

Byrne, T., LeSage, M. G., & Poling, A. (1997). Effects of chlorpromazine on rats' acquisition of lever-press responding with immediate and delayed reinforcement. *Pharmacology Biochemistry and Behavior, 58*(1), 31-35.

Cho, A. K., Hiramatsu, M., DiStefano, E. W., Chang, A. S., & Jenden, D. J. (1990). Stereochemical differences in the metabolism of 3,4-

methylenedioxymethamphetamine in vivo and in vitro: A pharmacokinetic analysis. *Drug Metabolism and Disposition, 18*, 686-691.

Commins, D. L., Vosmer, G., Virus, R. M., Woolverton, W. L., Schuster, C. R., & Seiden, L. S. (1987). Biochemical and histological evidence that methlylenedioxymethamphetamine (MDMA) is toxic to neurons in the rat brain. *Journal of Pharmacology and Experimental Therapeautics, 241*, 338-345.

Commons, M. L., Woodford, M., Boitano, G. A., Ducheney, J. R., & Peck, J. R. (1982). Acquisition of preference during shifts between terminal links in concurrent chain schedules. In M. L. Commons, R. J. Herrnstein, & A. R. Neuringer (Eds.), *Quantitative analyses of behavior: Acquisition* (Vol. 3, pp. 391-426). Cambride, MA: Ballinger.

Creighton, F. J., Black, D. L., & Hyde, C. E. (1991). Ecstasy psychosis and flashbacks. *British Journal of Psychiatry, 159*, 713-715.

Critchfield, T. S., & Lattal, K. A. (1993). Acquisition of a spatially defined operant with delayed reinforcement. *Journal of the Experimental Analysis of Behavior, 59*, 373-387.

Cuomo, M. J., Dyment, P. G., & Gammino, V. M. (1994). Increasing use of "Ecstasy" (MDMA) and other hallucinogens on a college campus. *Journal of American College Health, 42*, 271-274.

Dews, P. B. (1960). Free-operant behavior under conditions of delayed reinforcement. *Journal of the Experimental Analysis of Behavior, 3*, 221-234.

Dickinson, A., Watt, A., & Griffiths, W. J. H. (1992). Free-operant response acquisition with delayed reinforcement. *Quarterly Journal of Experimental Psychology, 45B*, 241-258.

Dowling, G. P., McDonough, E. T., & Bost, R. O. (1987). "Eve" and "Ecstasy": A report of five deaths associasted with the use of MDEA and MDMA. *Journal of the American Medical Association, 257*(12), 1615-11617.

Evans, E. B., & Wenger, G. R. (1992). Effects of drugs of abuse on acquisition of behavioral chains in squirrel monkeys. *Psychopharmacology, 107*, 55-60.

Frederick, D. L., Ali, S. F., Slikker, W., Gilliam, M. P., Allen, R. R., & Paule, M. G. (1995a). Behavioral and neurochemical effects of chronic

methylenedioxmethamphetamine treatment in rhesus monkeys. *Neurotoxicology and Teratology, 17*, 531-543.

Frederick, D. L., Gillam, M. P., Allen, R. R., & Paule, M. G. (1995b). Acute effects of methylenedioxymethamphetamine (MDMA) on several complex brain functions in monkeys. *Pharmacology Biochemistry and Behavior, 51*, 301-307.

Gold, L. H., Koob, G. F., & Geyer, M. A. (1988). Stimulant and hallucinogenic behavioral profiles of 3,4-methylenedioxymethamphetamine and N-ethyl-3,4-Methlyenedioxymethamphetamine in rats. *Journal of Pharmacology and Experimental Therapeutics, 247*, 547-555.

Green, A. R., Cross, A. J., & Goodwin, G. M. (1995). Review of the pharmacology and clinical pharmacology of 3,4-methlenedioxymethamphetamine (MDMA or "Ecstasy"). *Psychopharmacology, 119*, 247-260.

Greer, G., & Tolbert, R. (1986). Subjective reports of the effects of MDMA in a clinical setting. *Journal of Psychoactive Drugs, 18*, 319-327.

Henry, J. A., Jeffreys, K. J., & Dawling, S. (1992). Toxicity and deaths from 3,4-methylenedioxymethamphetamine ("Ecstasy"). *Lancet, 340*, 384-387.

Johnston, J. M., & Pennypacker, H. S. (1993). *Strategies and tactics of behavioral research.* Hilldale, NJ: Erlbaum.

Kamien, J. B., Johanson, C. E., Schuster, C. R., & Woolverton, W. L. (1986). The effects of (±)methylenedioxymethamphetamine and (±)methylenedioxyamphetamine in monkeys trained to discriminate (+)amphetamine from saline. *Drug and Alcohol Dependence, 18*, 139-147.

Klevin, M. S., Woolverton, W. L., & Seiden, L. S. (1989). Evidence that both intragastric and subcutaneuosly administered MDMA produce 5-HT neurotoxicity in rhesus monkeys. *Brain Research, 448*, 121-125.

Lamb, R. J., & Griffiths, R. R. (1987). Self-injection of 3,4-methylenedioxymethamphetamine (MDMA) in the baboon. *Psychopharmacology, 91*, 268-272.

Lattal, K. A., & Gleeson, S. (1990). Response acquisition with delayed reinforcement. *Journal of Experimental Psychology: Animal Behavior Processes, 16*, 27-39.

Lattal, K. A., & Metzger, B. (1994). Response acquisition by Siamese fighting fish (*Beta Splendens*) with delayed visual reinforcement. *Journal of the Experimental Analysis of Behavior, 61*, 35-44.

Lattal, K. A., & Williams, A. M. (1997). Body weight and response acquisition with delayed reinforcement. *Journal of the Experimental Analysis of Behavior, 67*, 131-143.

Lawn, J. C. (1986). Schedules of controlled substances; scheduling of 3,4-methylenedioxymethamphetamine (MDMA) into schedule I of the Controlled Substance Act. *Federal Register, 51*, 36552-36560.

LeSage, M., Clark, R., & Poling, A. (1993). MDMA and memory: the acute and chronic effects of MDMA in pigeons performing under a delayed-matching-to-sample procedure. *Psychopharmacology, 110*, 327-332.

LeSage, M., & Poling, A. (1997). MDMA and *d*-amphetamine produce comparable effects in pigeons performing under a multiple fixed-ratio interresponse-time-greater-than-t schedule of food delivery. *Pharmacology Biochemistry and Behavior, 57*, 173-177.

LeSage, M. G., Byrne, T., & Poling, A. (1996). Effects of *d* -amphetamine on response acquisition with immediate and delayed reinforcement. *Journal of the Experimental Analysis of Behavior, 66*, 349-367.

Lim, H. K., & Foltz, R. L. (1988). In vivo and in vitro metabolism of 3,4-(methylenedioxy)methamphetamine in the rat: Identification of metabolites using an ion trap detector. *Chemical Research in Toxicology, 1*, 370-378.

Michael, J. L. (1993). *Concepts and principles of behavior analysis*. Kalamazoo, MI: Society for the Advancement of Behavior Analysis.

Molliver, M. E., Berger, U. V., Mamounas, L. A., Molliver, D. C., O'Hearn, E., & Wilson, M. A. (1990). Neurotoxicity of MDMA and related compounds: anatomic studies. *New York Academy of Sciences, 600*, 640-664.

Nash, J. F., Meltzer, H. Y., & Gudelsky, G. A. (1990). Effect of 3,4-Methylenedioxymethamphetamine on 3,4-Dihydroxyphenylalanine accumulation in the striatum and nucleus accumbens. *Journal of Neurochemistry, 54*, 1062-1067.

Nichols, D. E. (1986). Differences between the mechanism of action of MDMA, MBDB, and the classic hallucinogens. Identification of a new therapeutic

class: Entactogens. *Journal of Psychoactive Drugs, 18*, 305-313.

Oberlender, R., & Nichols, D. E. (1988). Drug discrimination studies with MDMA and amphetamine. *Psychopharmacology, 95*, 71-76.

O'Brien, C. P. (1996). Drug addiction and drug abuse. In J. G. Hardman, L. E. Limbird, P. B. Molinoff, R. W. Ruddon, & A. G. Gilman (Eds.). *The pharmacologocal basis of therapeutics* (Ninth ed.,). New York: McGraw-Hill.

Paule, M. G., & McMillan, D. E. (1984). Incremental repeated acquisition in the rat: Acute effects of drugs. *Pharmacology Biochemistry and Behavior, 21*, 431-439.

Picker, M., & Negus, S. S. (1993). Drugs and stimulus control: Generalization, discrimination and threshold procedures. In F. vanHaaren (Ed.). *Methods of behavioral pharmacology* . New York: Elsevier.

Poling, A. (1986). *A primer of human behavioral pharmacology*. New York: Plenum.

Ricuarte, G. A., Bryan, G., Strauss, L., Seiden, L., & Schuster, C. (1985). Hallucinogenic amphetamine selectively destroys brain serotonin nerve terminals. *Science, 229*, 986-988.

Ricuarte, G. A., Finnegan, K. T., Irwin, I., & Langston, J. W. (1990). Aminergic metabolites in cerebrospinal fluid of humans previously exposed to MDMA: Preliminary observations. *Annals of the New York Academy of Science, 600*, 699-710.

Ricaurte, G. A., Forno, L. S., Wilson, M. A., DeLanney, L. E., Irwin, I., Molliver, M. E., & Langston, J. W. (1988). (±) 3,4-Methylenedioxymethamphetamine selectively damages central serotonergic neurons in non-human primates. *Journal of the American Medical Association, 260*, 51-55.

Ricuarte, G. A., Markowska, A. L., Wenk, G. L., Hatzidimitriou, G., Wlos, J., & Olton, D. S. (1993). 3,4-Methylenedioxymethamphetamine, serotonin and memory. *Journal of Pharmacology and Experimental Therapeautics, 266*, 1097-1105.

Sabol, K. E., Lew, R., Richards, J. B., Vosmer, G. L., & Seiden, L. S. (1996). Methlenedioxymethamphetamine-induced serotonin deficits are followed by partial recovery over a 52-week period. Part 1: Synaptosomal uptake and

tissue concentrations. *Journal of Pharmacology and Experimental Therapeautics, 276*, 846-854.

Saunders, N., & Doblin, R. (1996). *Ecstasy: Dance trance & transformation.* Oakland, California: Quick American Archives.

Scanzello, C. R., Hatzidimitriou, G., Martello, A. L., Katz, J. L., & Ricuarte, G. A. (1993). Response of central serotonergic neurons to (±)3,4-methlenedioxymethamphetamine (MDMA) injury: Observations in rats. *Journal of Pharmacology and Experimental Therapeautics, 264*, 1484-1491.

Schechter, M.D. (1989). Seronergic-dopaminergic mediation of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *Pharmacology, Biochemistry & Behavior, 31*, 817-824.

Schechter, M. D. (1991). Effect of MDMA neurotoxicity upon its conditioned place preference and discrimination. *Pharmacology Biochemistry & Behavior, 38*, 539-544.

Schechter, M. D. (1998). MDMA-like stimulus effects of hallucinogens in male fawn-hooded rats. *Pharmacology Biochemistry and Behavior, 59*, 265-270.

Schlinger, H. D., & Blakely, E. (1994). The effects of delayed reinforcement and a response-produced auditory stimulus on the acquisition of operant behavior in rats. *The Psychological Record, 44*, 391-409.

Skinner, B. F. (1953). *Science and human behavior*. New York: Macmillan.

Spanos, L. J., & Yamamoto, B. K. (1989). Acute and subchronic effects of methylenedioxymethamphetamine [(±)MDMA] on locomotion and serotonin syndrome behavior in the rat. *Pharmacology biochemistry & Behavior, 32*, 835-840.

Steele, T. D., McCann, U. D., & Ricuarte, G. A. (1994). 3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy"): pharmacology and toxicology in animals and humans. *Addiction, 89*, 539-551.

Stone, D. M., Johnson, M., Hanson, G. R., & Gibb, J. W. (1988). Role of endogeneous dopamine in the central serotonergic deficits induced by 3,4-methylenedioxymethamphetamine. *Journal of Pharmacology and Experimental Therapeutics, 247*, 79-87.

Stone, D. M., Stahl, D. C., Hansen, G. R., & Gibb, J. W. (1986). The effects of 3,4-

methylenedioxymethamphetamine (MDMA) and 3,4-methlyenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain. *European Journal of Pharmacology, 128*, 41-48.

Substance Abuse and Mental Health Services Administration (1996). Preliminary results from the 1996 National Household Survey on Drug Abuse.

Sutphin, G., Byrne, T., & Poling, A. (1998). Response acquisition with delayed reinforcement: A comparison of two-lever procedures. *Journal of the Experimental Analysis of Behavior, 69*, 17-28.

Thompson, D. M. (1974). Repeated acquisition of response sequences: Effects of *d*-amphetamine and chlorpromazine. *Pharmacology Biochemistry and Behavior, 2*, 741-746.

Thompson, D. M., Winsauer, P. J., & Mastropaolo, J. (1987). Effects of phencyclidine, ketamine, and MDMA on complex operant behavior in monkeys. *Pharmacology biochemistry and behavior, 26*, 401-405.

van Harren, F. (1992). Response acquisition with fixed and variable resetting delays of reinforcement in male and female Wistar rats. *Physiology and Behavior. 52*, 769-772.

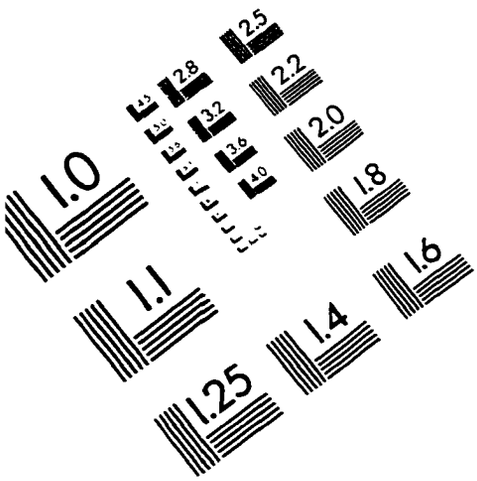
Wilkenfield, J., Nickel, M., Blakely, E., & Poling, A. (1992). Acquisition of lever-press responding in rats with delayed reinforcement: A comparison of three procedures. *Journal of the Experimental Analysis of Behavior, 58*, 431-443.

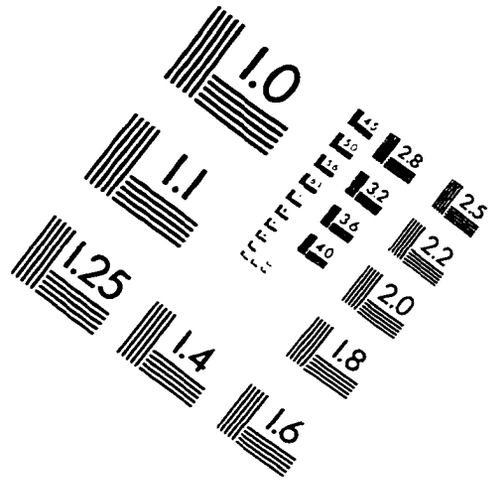
Zeiler, M. D. (1971). Eliminating behavior with reinforcement. *Journal of the Experimental Analysis of Behavior, 16*, 401-405.

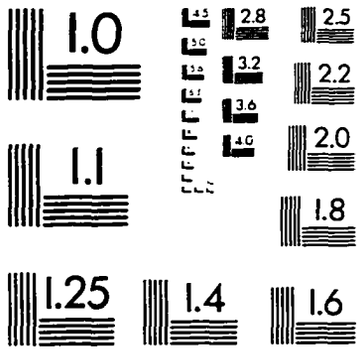
Zeiler, M. D. (1976). Positive reinforcement and the elimination of reinforced behavior. *Journal of the Experimental Analysis of Behavior, 26*, 37-44.

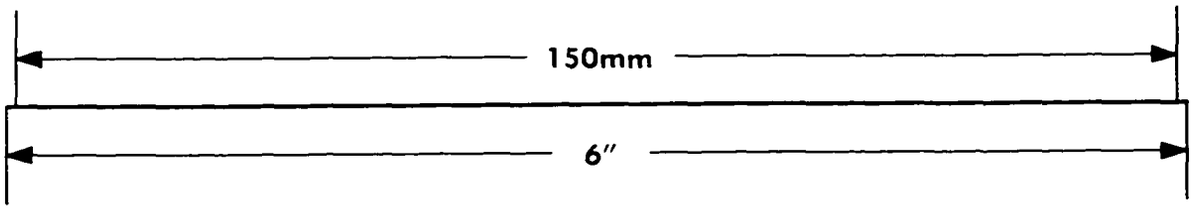
Zeiler, M. D. (1979). Reinforcing the absence of fixed-ratio performance. *Journal of the Experimental Analysis of Behavior, 31*, 321-332.

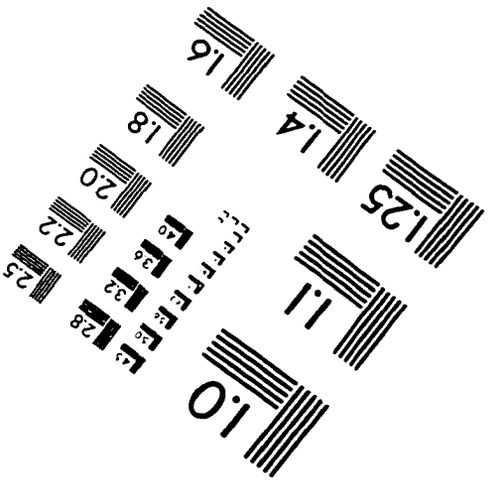
IMAGE EVALUATION TEST TARGET (QA-3)

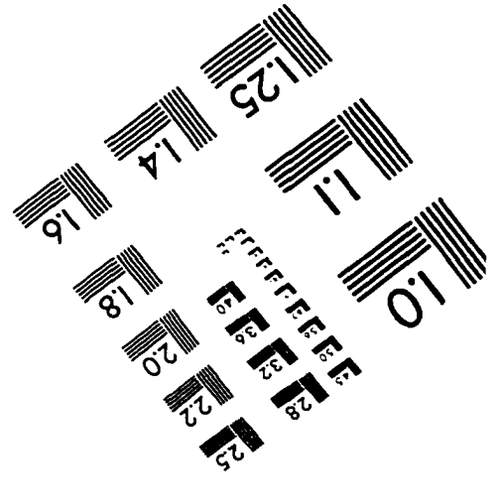












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989