12-2004

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REINFORCEMENT SCHEDULES MODULATE DISCRIMINATIVE
STIMULUS PROPERTIES OF
3,4-METHYLENEDIOXYMETHAMPHETAMINE
AND COCAINE

by

Daniel Kueh

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Arts
Department of Psychology

Western Michigan University
Kalamazoo, Michigan
December 2004

REINFORCEMENT SCHEDULES MODULATE DISCRIMINATIVE STIMULUS PROPERTIES OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE AND COCAINE

Daniel Kueh, M.A.

Western Michigan University, 2004

Drug discrimination is a model used to assess the subjective effects of different psychoactive drugs such as 3,4-methylenedioxymethamphetamine (MDMA) and cocaine. However, results from MDMA discrimination studies across different laboratories have not been consistent. Possible confounds for this inconsistency may include the use of different reinforcement schedules such as the fixed-ratio 20 (FR20) and the variable interval 15 seconds (VI15 s) during discrimination training. Studies examining the effects of these two schedules on the discriminative stimulus properties of MDMA and cocaine have not been conducted. Thus, the present study compared the FR20 and the VI15 s schedules to determine their influence on discrimination acquisition, response rates, frequency of reinforcements and stimulus generalization in rats trained to discriminate cocaine (10 mg/kg) or MDMA (1.5 mg/kg) from saline. Compared to the VI15 s schedule, the FR20 schedule facilitated rapid discrimination acquisition and established differential response rates and frequency of reinforcement under drug and vehicle conditions. MDMA ($ED_{50} = 0.75$ mg/kg) was also found to substitute for cocaine in rats trained to discriminate cocaine from saline.

ACKNOWLEDGMENTS

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INTRODUCTION

Drug discrimination is a tool used by many behavioral pharmacologists as a model to classify psychoactive drugs, to compare novel and existing drugs, and to determine neurochemical actions that mediate the discriminative stimulus properties of drugs. The procedure typically involves training an animal to respond in the presence of one drug and to respond differently in the presence of another drug or vehicle condition. Training is done repeatedly until the animal has met a set criterion and is then exposed to different doses of a novel or training drug under abbreviated periods of time. If the animal emits a drug-designated response in the presence of the novel drug, stimulus generalization between the training and novel drug has occurred. Conversely, if the animal emits a vehicle-designated response, stimulus generalization between the two drugs has not occurred. In the latter instance, both drugs are concluded to have different discriminative stimulus properties even though both drugs may be similar in chemical structure.

As an assay, drug discrimination has been used to test a variety of different psychoactive substances (e.g. Stephens, Schneider, Kehr, Jensen, Petersen, & Honore, 1987; Balster, & Prescott, 1992; Browne, 1986). It is popular because it is assumed that the discriminative stimulus properties of drugs parallel the subjective effects of the same drugs in humans (Goudie & Leathley, 1993). Knowledge of the discriminative stimulus properties of drugs therefore has predictive value with respect to understanding the abuse potential of specific drugs. Moreover, the procedure itself has shown to be a stable, specific, and sensitive measure of dose-related drug effects (Glennon, Rosecrans, & Young, 1983).

A variety of different approaches and techniques have been used in drug discrimination studies. Methodological approaches that differ across different laboratories include the type of apparatus, reinforcement schedules, species, sample size, reinforcers, and dose-response testing procedures (e.g., Colombo, Agabio, Balaklievskaia, Lobina, Reali, Fadda, & Gessa, 1996; Schechter, 1997). Most investigators however, employ a standard two lever operant chamber with a sample size of six to eight rats. The two popular reinforcement schedules used by investigators are fixed–ratio (FR) schedules (e.g., Goodwin, Pynnonen, & Baker, 2003; Broadbent, Michael, & Appel, 1989; Appel, West, Rolandi, Alici, Pechersky, 1999) and variable interval (VI) schedules (e.g. Glennon, & Young, 1984). A survey by Stolerman (1989) on 606 drug discrimination studies that were published between 1951 and 1986 alone showed that 396 studies used FR schedules of reinforcement and 115 studies used VI schedules of reinforcement.

The FR schedule requires a subject to emit a fixed number of consecutive responses before reinforcement. To ensure that responding is fixed and consecutive in a two lever condition, the FR schedule has a resetting component that punishes nonconsecutive responding by requiring subjects to repeat the required number of responses before reinforcement. The VI schedule however, is dependent on the first response made after the passage of a variable interval of time. The number of variable intervals in this schedule is arbitrary as long as two conditions are met: 1) the progression of variable intervals is random over time and 2) the average of these intervals is consistent across different sessions. For example, a VI 15 s can have twenty intervals that range from 1-60 seconds, which are then randomly selected over

time in each individual training session. However, the average of the twenty intervals selected randomly is 15 seconds and is the same across different training sessions. Thus, VI schedules generally preclude any use of a timeout or resetting component as in the case of this example, as it would affect the average value of the twenty intervals.

The use of different reinforcement schedules in drug discrimination is not without justification however, since each schedule easily lends itself to either a quantal or a quantitative measurement of the discriminative stimulus properties of drugs (Barrett, R., Caul, W., Huffman, E., & Smith., R., 1994). Quantal measures are nominal dose-response measures in which subjects are assumed to "perceive" the drug cue in an all-or-none fashion following drug treatment. Thus, a decision on whether a test drug actually substitutes for the training drug is based on the number of subjects selecting the drug lever during substitution testing. If most of the animals selected the drug lever, then it is concluded that substitution of the test drug for the training drug has occurred. Conversely, if most of the animals selected the vehicle lever, then it is concluded that substitution of the test drug for the training drug did not occur.

With quantal measures, the data are typically presented as a percentage of animals that emit drug-designated responses that equal the FR requirement or drug-designated response that equal or exceed 50%. The choice of 50% as the threshold is based on the assumption that responding is a continuous function of stimulus similarity (Mathis, & Emmett-Oglesby, 1990). Thus, a test drug with stimulus properties of intermediate similarity to the training drug would evoke responses to

both levers, leading to responding that is evenly distributed across both levers. Thus, quantal interpretations generally preclude classifying drug cues on a continuum based on intensity and quality. Such an approach is popular among several drug discrimination investigators because it eliminates the difficulties of interpreting *partial generalizations* in drug substitution testing, as would be the case when using the quantitative measurement. The FR schedule is therefore convenient for this purpose since FR-trained animals respond in "bursts" that equal the schedule requirement, i.e., there is no switching back and forth between levers as FR schedule amplifies preference.

In the quantitative measurement, it is assumed that the discriminative stimulus properties of drugs vary along a single dimension such as the intensity or quality of the drug. The measure itself is based on an interval or ordinal scale (Stolerman, 1991). Responding to drug cues is therefore graded and not an all-or-none event as would be the case when the quantal measure is used. Variable interval (VI), variable ratio (VR), or fixed interval (FI) schedules are suitable for quantitative measurements since subjects are not required to make fixed consecutive responses during substitution testing. Quantitative data are plotted as the percentage of drug-appropriate responses divided by the total number of drug and vehicle responses. Quantitative analysis therefore allows for classification of drug cues on a continuum based on the intensity or quality of the drug.

Several studies have examined the influence of reinforcement schedules on drug discrimination (e.g., Snodgrass, & McMillan, 1991). For example, Snodgrass and McMillan (1991) investigated the influence of FR and FI schedules on

pentobarbital discrimination in rats. They reported that responses under the FR schedule occurred more steadily than responses under the FI schedule and more response errors occurred under the FI schedule compared to the FR schedule. Their results are consistent with previous findings demonstrating that reinforcement schedules determine the degree of stimulus control by the training drug (Overton, 1979).

Since different reinforcement schedules can modulate the discriminative stimulus properties of drugs, it is possible that inconsistent results obtained from different laboratories are due to the different reinforcement schedules that were used. For example, Glennon and Young (1984) reported that (±)-3,4-methylenedioxymethamphetamine (MDMA) produced complete substitution for amphetamine in rats trained to discriminate amphetamine from saline. Their results were inconsistent with Oberlender and Nichols's report that (±)-MDMA did not substitute for amphetamine (Oberlender & Nichols, 1988). Oberlender et al. (1988) trained rats under an FR50 schedule of food reinforcement, whereas Glennon and Young (1984) trained rats under a VI15 s schedule of food reinforcement.

In a recent study, Khorana et al. compared the discriminative stimulus properties of cocaine and MDMA (Khorana, Pullagurla, Young, & Glennon, 2004). Using a VI15 s schedule as the training schedule, they found S(+)MDMA, R(—)MDMA, and cocaine substituted for (±)MDMA in rats trained to discriminate (±)MDMA from saline. However, when S(+)MDMA, R(—)MDMA, and (±)MDMA were tested on cocaine-trained animals, substitution for cocaine did not take place. Thus, they concluded that stimulus generalization

between MDMA and cocaine to be asymmetrical, i.e., cocaine substituted for MDMA but MDMA did not substitute for cocaine.

The results of Khorana et al. (2004) are inconsistent with those of an earlier study by Schechter (1998). Using the FR10 schedule of reinforcement as the training schedule, Schechter reported that cocaine did not substitute for MDMA in Fawn-Hooded rats trained to discriminate MDMA from saline. Khorana et al. (2004) explained this apparent inconsistency by pointing to the different methodological approaches and different strains of animals used in both studies. Because both studies employed different reinforcement schedules and testing procedures, the different procedures themselves may also have affected the obtained results in those two studies.

Although a number of studies have compared the effects of different types of reinforcement schedules used in drug discrimination, none have been conducted with either MDMA or cocaine, two commonly abused drugs by young adults (Yacoubian, 2002). MDMA is a synthetic amphetamine derivative whereas cocaine is a nonsynthetic alkaloid found in the coca leaves. Both drugs share related neurochemical effects by increasing synaptic levels of dopamine (DA) and serotonin (5-HT) (Ritz, Lamb, Goldberg, & Kuhar, 1987; Shulgan, 1986). However, their mechanisms of action differ as cocaine inhibits the reuptake of DA whereas MDMA induces the release of 5-HT as well as blocking its reuptake into the nerve terminal (Ritz et al., 1987; Shulgan, 1986). A comparison of different reinforcement schedules with MDMA and cocaine would not only address previously discussed

inconsistent results, but would also further our understanding of the discriminative stimulus properties of MDMA relative to cocaine.

Thus, to understand the influence of two commonly used reinforcement schedules, FR 20 and VI 15 s, on the discriminative stimulus properties of MDMA and cocaine, two experiments were conducted. The first experiment involved training two groups of rats to discriminate cocaine from saline using either an FR20 or VI15 s schedule, respectively. The second experiment involved training two groups of rats to discriminate MDMA from saline that were also maintained under the FR20 or VI15 s schedules.

The first aim of the two current experiments was to compare discrimination acquisition of MDMA and cocaine under the FR20 and VI 15 s schedule of reinforcement. The second aim was to compare other dependent measures such as response rates and frequency of reinforcement. The third aim was to compare dose-response curves of MDMA and cocaine for two groups of rats in the first experiment that were maintained under FR20 and VI15 s schedules, with cocaine as their training drug. Dose-response curves were generated using both quantal and quantitative measures. The fourth aim was to compare dose-response curves that were obtained using both discrete and cumulative dose-response procedures.

METHOD

Subjects

Thirty-two male Sprague-Dawley rats (Charles River Laboratories, Portage, MI), 50 to 60 days old were used. They were acclimated to an animal colony maintained on a 12 h light/12 h dark cycle at a constant temperature ($20°$ C $\pm$ $2°$ C) and humidity (50% $\pm$ 5%) for a week and were housed in individual Plexiglas cages. All thirty-two rats were food deprived to approximately 80-85% of their free-feeding weights, but received water *ad libitum*. Subjects were maintained according to the general principles of animal husbandry as stated by the US Department of Health, Education, and Welfare (National Research Council, 1996). The Institutional Animal Care and Use Committee (IACUC) of Western Michigan University (WMU) approved the research protocol.

Apparatus

Drug training and testing were conducted using eight operant conditioning chambers (MED Associates Inc., Georgia, VT), measuring 28 cm long by 21 cm wide by 21 cm high. Each chamber contained two retractable levers, a food receptacle, a 28-V white light to illuminate chamber, and a fan to mask noise and provide ventilation. A minimum force of 0.14 N was required to operate levers. Food pellets (Bio-serv #F0021, Frenchtown, NJ) weighing 45 mg each served as reinforcers. Programming and data event recordings were done using MED-PC software installed on an IBM-compatible computer.

Drug

Both (±)-MDMA-hydrochloride and (−)-cocaine-hydrochloride were obtained from the National Institute on Drug Abuse (Rockville, MD). The drugs were dissolved in 0.9 % bacteriostatic sodium chloride and were administered by intraperitoneal (IP) injection using Monoject insulin syringes (Sherwood Medical, St. Lois, MO) 15 minutes before each session. Drug doses were calculated based on the weight of the salt.

Discrimination Training and Reinforcement Schedules

Subjects were divided into four groups through random assignment. In Experiment 1, Group A was trained under an FR 20 schedule of reinforcement with cocaine (10 mg/kg) as the training drug. Group B was trained under a VI 15 s schedule of reinforcement with cocaine as the training drug (10 mg/kg). In Experiment 2, Group C was trained under an FR 20 schedule of reinforcement with MDMA (1.5 mg/kg) as the training drug. Group D was trained under a VI 15 s schedule of reinforcement with MDMA (1.5 mg/kg) as the training drug.

Subjects were first exposed to an hour of fixed time 60 s (FT 60 s) schedule of food delivery without the levers present, to pair the sound of pellet drop with food availability. Food delivery was not contingent on any response. Following this procedure, eight lever press training sessions were conducted whereby all subjects were exposed to an errorless training condition. Under this condition, only one of two levers was present, depending on whether drug or vehicle was administered. Subjects received four days of errorless training under drug condition and four days of errorless training under vehicle condition.

Groups A and B (10 mg/kg) received IP injections of cocaine fifteen minutes before training, whereas Groups C and D received IP injections of MDMA (1.5 mg/kg), also at fifteen minutes before training. For the first half of the animals trained under the FR20 schedule, the right lever was designated as drug-appropriate and the left lever was designated as vehicle-appropriate. This designation was reversed for the first half of animals trained under the VI 15 s schedule. Lever assignments were reversed for the remaining animals in each group.

After the errorless training conditions, training of all subjects began on an FR1 schedule with both levers present. Isopropyl alcohol was used to wipe both levers before each training session to control for possible olfactory cues (Extance, & Goudie, 1981). Once subjects were reliably responding on the levers, subsequent training was conducted under an FR 20 schedule for Groups A and C and a VI 15 s schedule for groups B and D.

Subjects in Groups A and C trained under the FR20 schedule were first exposed to an initial value of FR1, which incremented progressively to a resetting FR20 schedule. Depending on each subject's performance, the starting FR value per session was automatically incremented by "n" after every 5th reinforcement where "n" was any number from 1-19. The starting FR value and increment value were the same for all subjects and were systematically increased across sessions until all subjects were responding reliably under an FR20 schedule under both drug and vehicle conditions. For reinforcement to occur, fixed consecutive responses on the correct lever were required as a single incorrect response would reset the response counter.

A VI15 s schedule arranged with 20 interresponse intervals (IRI) averaging 15 s was used to train subjects in groups B and D. The twenty IRIs used in progression were generated logarithmically based on the equation developed by Flescher and Hoffman (1962). Under this schedule, the first IRI was set at 16.874 seconds and was the same for all subjects. After the first reinforcement, the twenty IRIs were randomized and each subject was exposed to a different IRI progression. An IRI that was in effect at the given moment had to elapse before reinforcement of the first correct response occurred. Moreover, the IRI that was in effect did not progress to the next IRI until the first response on the correct lever was made. Incorrect lever presses had no programmed consequences.

Subjects were trained six days a week, and in the order of VVDDVD, VVDVDD, DDVVDV, or DDVDVV, whereby "D" is the drug session and "V" is the vehicle session. Each training session, regardless of schedule, lasted 15 minutes.

Substitution Testing

Cumulative Dose-Response Test. Once Group A and Group B animals had reached discrimination criterion, cumulative dose-response tests were conducted. Under this procedure, each test consisted of four trials, with each trial lasting 2.5 minutes under extinction conditions. The test trial also recorded the number of responses on both levers in five 30 seconds bins. This feature made possible the assessment of response rates and trends in probing responses within each trial.

Four doses of MDMA (0.375, 0.375, 0.75, and 1.5 mg/kg) and cocaine (1.25, 1.25, 2.5, and 5 mg/kg) were used. In the first trial, Group A and Group B subjects were administered the lowest dose of MDMA (0.375 mg/kg) or cocaine (1.25 mg/kg),

15 minutes before each trial. Once the trial ended, subjects were returned to their home cages and were again treated with a second dose of MDMA (1.25 mg/kg) or cocaine (0.375 mg/kg). The duration between each dose treatment was 20 minutes. This procedure was again repeated for the next two trials. Thus, by the end of the fourth trial, all subjects were exposed to a cumulative dose of MDMA (3.0 mg/kg) or cocaine (10 mg/kg). Both MDMA and cocaine were tested on two days separated by a week of regular discrimination training. The number of responses on each dose for each subject was therefore averaged across both test days.

Before being exposed to the cumulative doses of MDMA and cocaine, each group underwent the same testing procedure four times with three consecutive injections of saline followed by an injection of a training drug. This was done to ensure that lever presses were stable across multiple trials and to establish a baseline for drug-appropriate responding under saline conditions.

Discrete Dose-Response Test. Unlike the cumulative dose-response test, discrete dose-response tests were limited to every third day and to a single specified dose of MDMA or cocaine. On the intervening two days between each test, all animals were trained on vehicle and drug. Like the cumulative dose-response procedure, cocaine-trained animals received 0.375, 0.375, 0.75, and 1.5 mg/kg of MDMA, whereas MDMA-trained animals received 1.25, 1.25, 2.5, and 5 mg/kg of cocaine.

Data Analysis

Discrimination Acquisition. For all groups, numbering of training sessions began with the initiation of the FR1 training schedule with both levers present.

Group differences within a training drug were compared using a two-sample t-test with $p < .05$ being regarded as significant. Animals that did not meet criterion were not included in statistical analyses.

Response Rates and Frequency of Reinforcement. For response rates, the data of interest were the total number of responses made on the training drug-designated lever and the vehicle-designated lever under both FR20 and VI15 s schedules. Response rates were expressed as the number of responses per second (RPS). Group means were also calculated for each dose.

Stimulus Generalization. Data were presented as both quantal and quantitative measurements. The quantal measure was based on the percent of animals that emitted 50% or more drug-appropriate responses. The quantitative measure was based on the number of drug-appropriate responses divided by the total number of responses on both levers. For both quantal and quantitative measures, an animal had to make ≥ five responses during the entire test session to be included in the dose-response curve.

For both discrete and cumulative dose-response measures using quantal analyses, full substitution for MDMA and cocaine was defined as 80% or more subjects making at least 50% or more of their response on the drug-designated lever. With quantitative analyses however, full substitution for MDMA and cocaine using both discrete and cumulative dose-response was defined as the total number of drug-designated responses divided by the total number of drug-designated and vehicle-designated responses multiplied by 100%, and that is at least 80% and above. Moreover, partial substitution was defined as 20% < DL < 80%, whereby DL is

percent drug-appropriate responding and no substitution was defined as $\leq 20\%$ drug-appropriate responses.

For both quantal and quantitative measures, effective dose 50 (ED_{50}) values on dose-response curves were calculated and analyzed using non-linear regression analysis. All data analyses, including ED50 values, were calculated and graphed using GraphPad Prism 4 (GraphPad Software, Inc., San Diego, CA).

RESULTS

Discrimination Acquisition

The mean number of training sessions required for Group A to discriminate accurately ($\geq$80% on conditioned-appropriate lever for eight of the ten most recent sessions) was 40.4 $\pm$ 6.19 sessions (range 20-70 sessions, n=8), while the mean number of sessions for Group B was higher at 75.7 $\pm$ 18.38 sessions (range 31-154, n=7). With rats trained to discriminate 1.5 mg/kg MDMA from vehicle, Group C acquired discrimination after 60.5 $\pm$ 7.37 sessions (range 23-80 sessions, n=8), while the mean number of sessions for Group D was 99.4 $\pm$ 13.8 sessions (range 77-141 sessions, n=5). Two subjects in Group D have failed to meet criterion and were therefore excluded from statistical analyses for this measure. Bar graphs in Figure 1 refer to the four groups of rats that have met the discrimination criterion. Mean differences in cocaine-designated responding between Groups A and B were statistically significant in a two-tailed unpaired t-test, t(14) = 2.47, p = 0.0267. Mean differences in MDMA-designated responding between Groups C and D were also statistically significant in a two-tailed unpaired t-test, t(12) = 3.34, *p* = 0.0059.

Figures 2 and 3 show four discrimination acquisition curves for Groups A and B, and Groups C and D respectively. For subjects in Groups A and B trained under the FR 20 and VI 15 s schedules respectively, s*essions* in Figure 2 refer to the *n*th exposure to either cocaine or saline. Likewise, in Figure 3, *sessions* also refer to the *n*th exposure to either MDMA or saline for Groups C and D respectively.
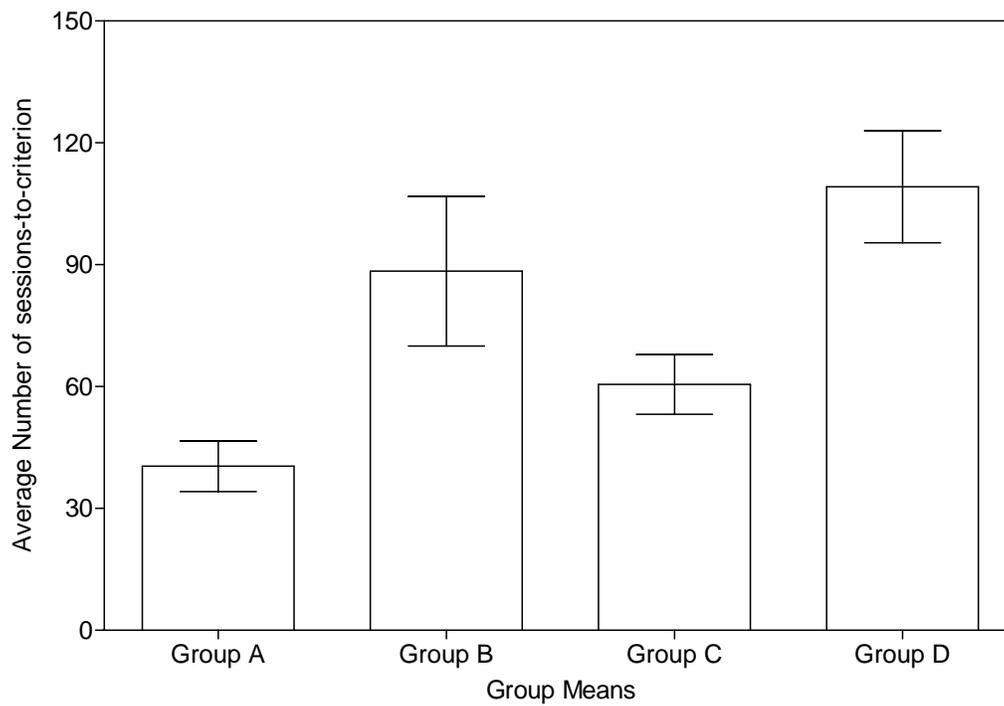
Figure 1. Mean number of sessions-to-criterion. Each bar represents a particular group of animals trained under a specified drug and reinforcement schedule. Vertical lines in each bar depict standard errors (SEM).
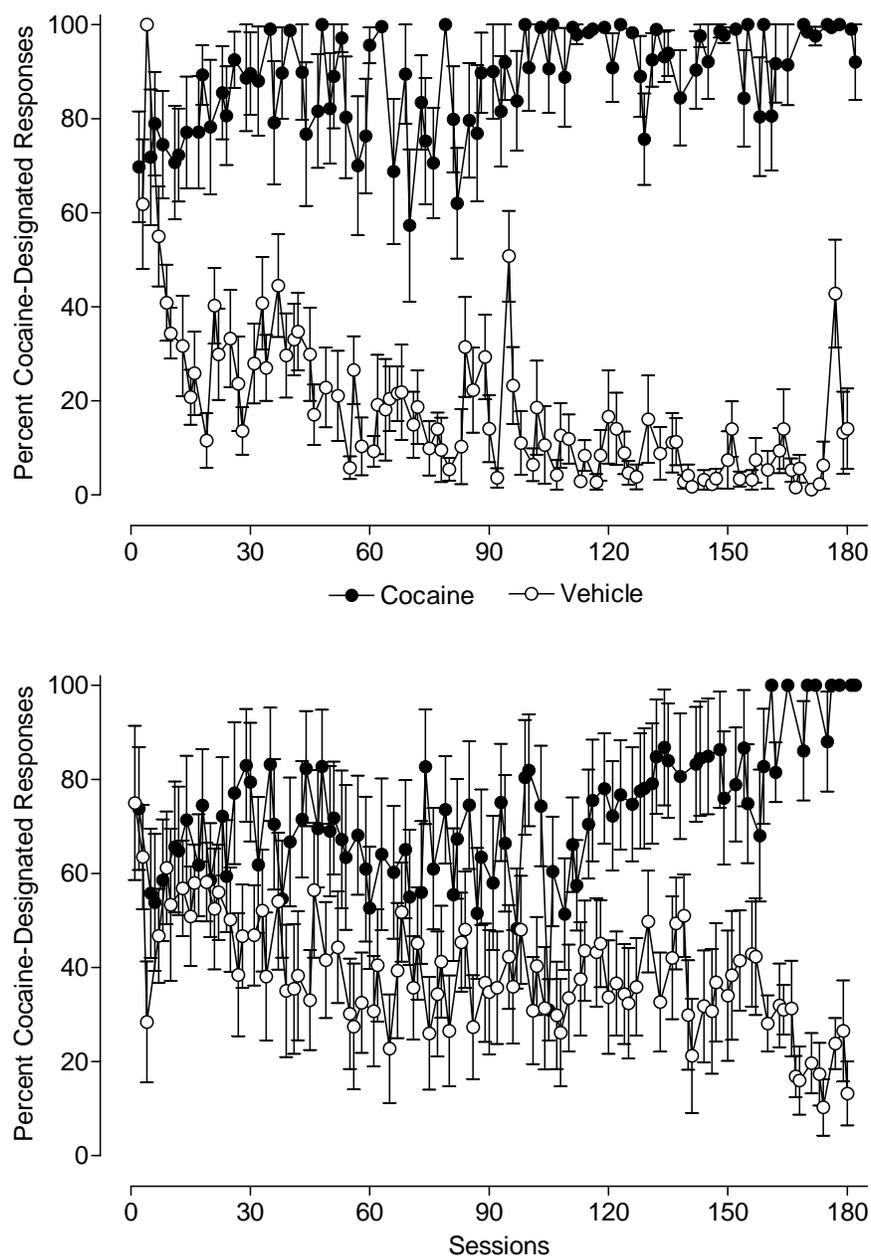
Figure 2: Percent cocaine-designated responses before first reinforcement for groups

A (upper graph) and B (lower graph). Points represent percent drug-designated

responses (n = 8 for Groups A and B). Vertical lines depict standard errors (SEM).
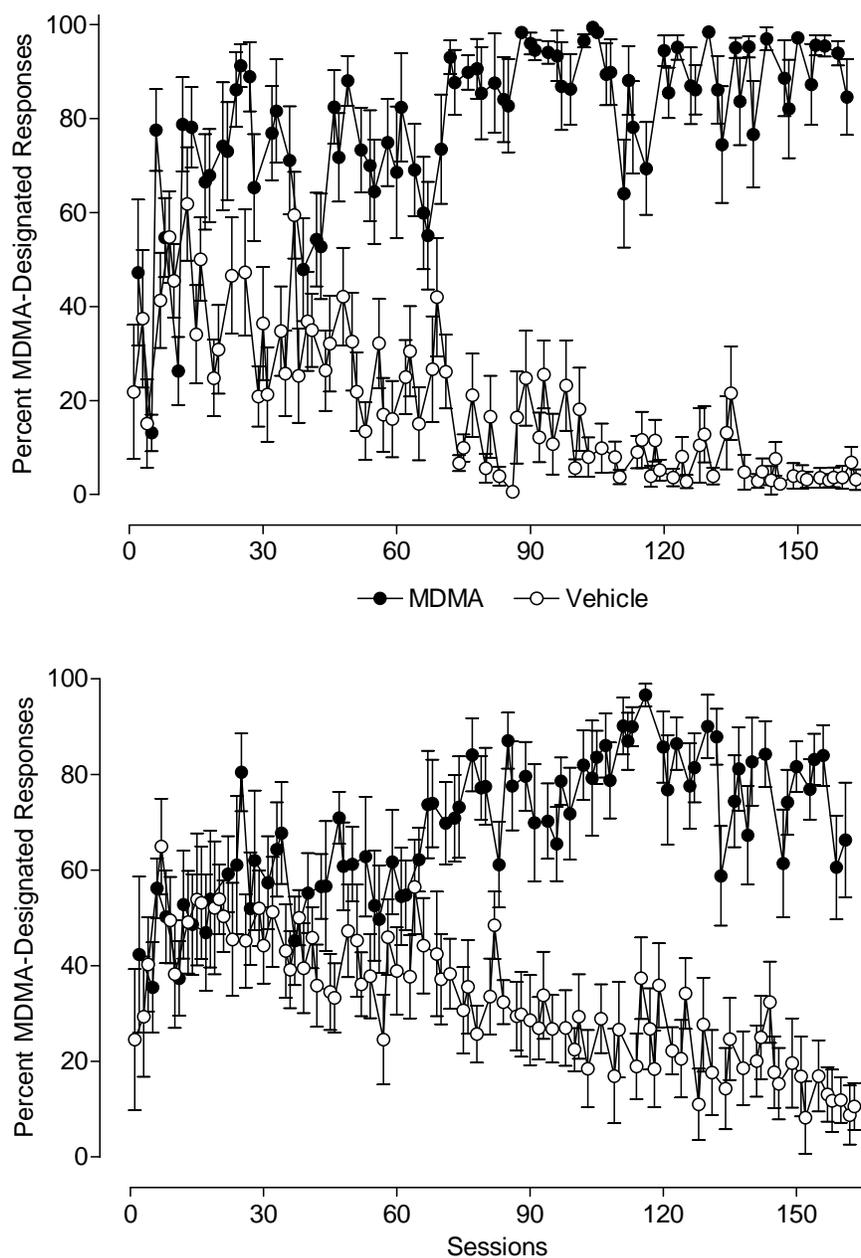
Figure 3: Percent MDMA-designated responses before first reinforcement for Group
C (upper graph) and Group D (lower graph). Points represent percent drug-
designated responses (n = 8 for group C and n = 6 for group D). Vertical lines depict
standard errors (SEM).

Percentages in Figures 2 and 3 were based on drug-appropriate responding before the first reinforcement under both FR 20 and VI 15 s schedules.

Response Rates and Frequency of Reinforcement

The mean values for response rate for Group A were 1.279 ± 0.042 RPS (mean $\pm$ *SEM*) and 1.686 ± 0.053 RPS under cocaine and vehicle conditions respectively. For Group B the mean values for response rate were lower than Group A at 0.932 ± 0.021 RPS and 1.026 ± 0.020 RPS under cocaine and vehicle conditions respectively. For group C, the mean values for response rate were 1.162 ± 0.033 RPS and 1.321 ± 0.031 RPS under MDMA and vehicle conditions respectively. For group D, the mean values for response rate were lower than group C at 0.691 ± 0.018 RPS and 0.846 ± 0.0192 RPS under MDMA and vehicle conditions respectively. Figures 4 and 5 depict response rates for Groups A, B, C, and D. Like figures 2 and 3, sessions refer to the *n*th exposure to either drug or saline condition.

The mean values for number of reinforcers earned by animals in Group A were 56 ± 1.75 (mean $\pm$ *SEM*) reinforcers and 75 ± 2.28 reinforcers under cocaine and saline conditions respectively. For Group B, the mean values for number of reinforcers earned were 54 ± 0.487 reinforcers and 55 ± 1.480 reinforcers under cocaine and vehicle conditions respectively. The mean values for number of reinforcers earned by animals in group C were 50 ± 1.39 reinforcers and 58 ± 1.32 reinforcers under MDMA and vehicle conditions respectively. For group D, the mean values for number of reinforcers earned were lower at 52 ± 0.506 reinforcers and $53 \pm$
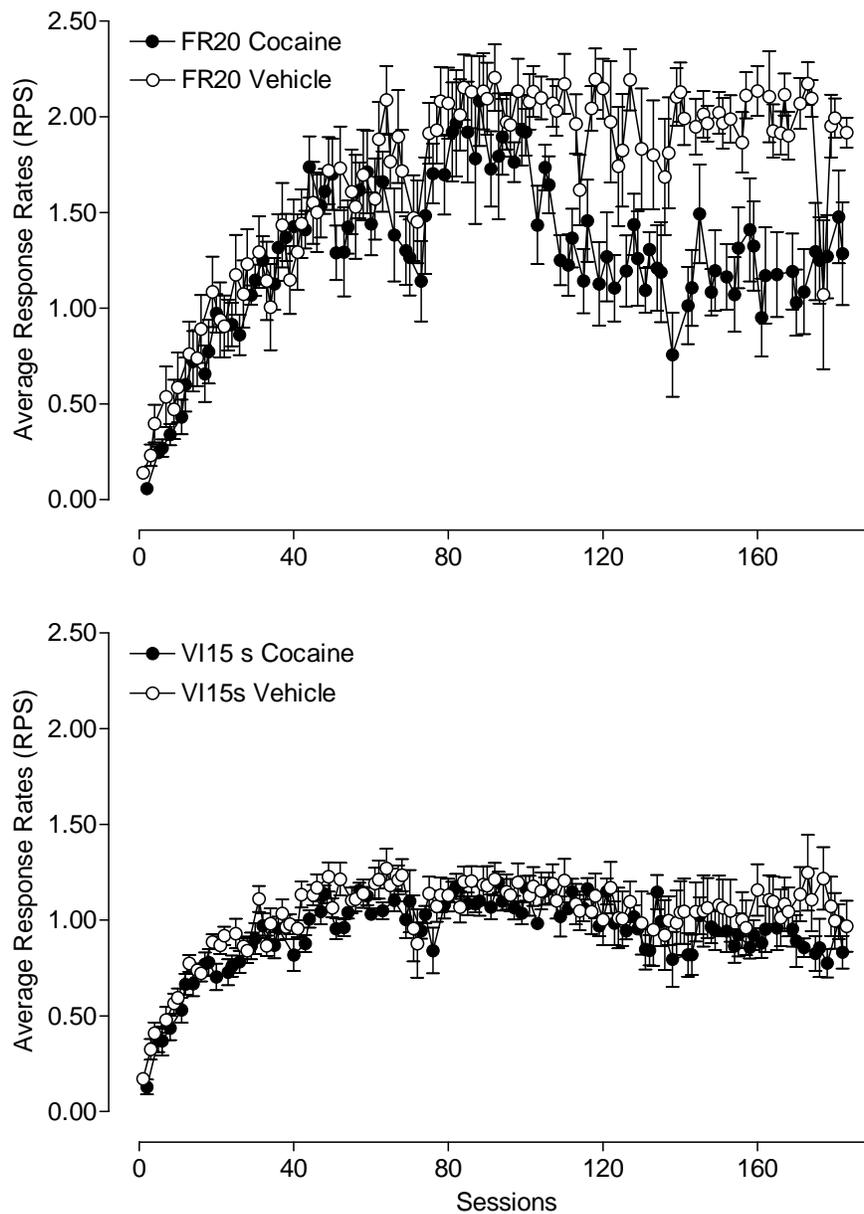
Figure 4: Average response rates for cocaine-trained rats in Groups A (upper graph)

and B (lower graph). Points represent average response rates; (n = 8 for Group A and

B). Vertical lines depict standard errors (SEM).
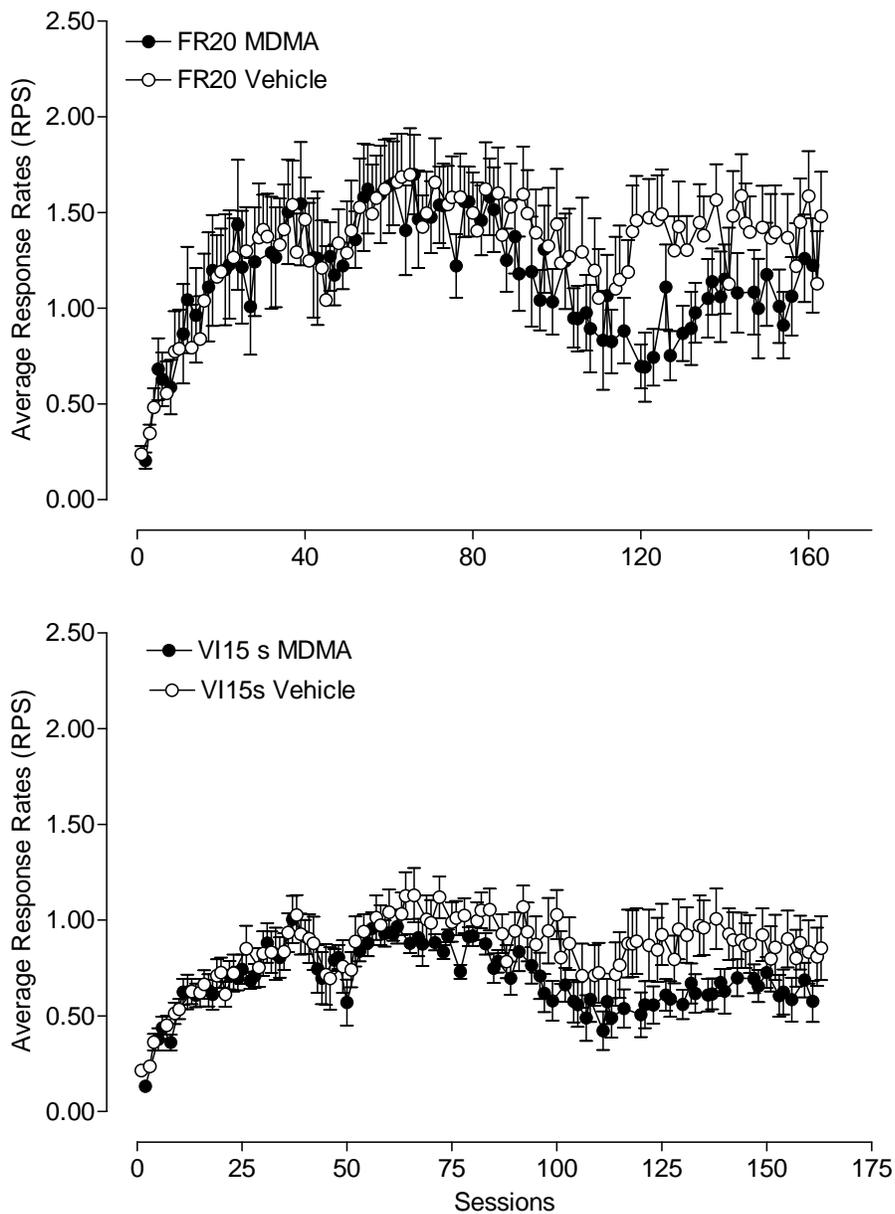
Figure 5: Average response rates for MDMA-trained rats in Groups C (upper graph)

and D (lower graph). Points represent average response rates; (n = 8 for group C and

D). Vertical lines depict standard errors (SEM).

Figure 6. Average number of reinforcers earned by cocaine-trained rats under drug

and vehicle conditions in both FR20 (upper graph) and VI15 s schedules (lower

graph). Points represent mean total number of reinforcers (n = 8 for Groups A and

B). Vertical lines depict standard errors (SEM).
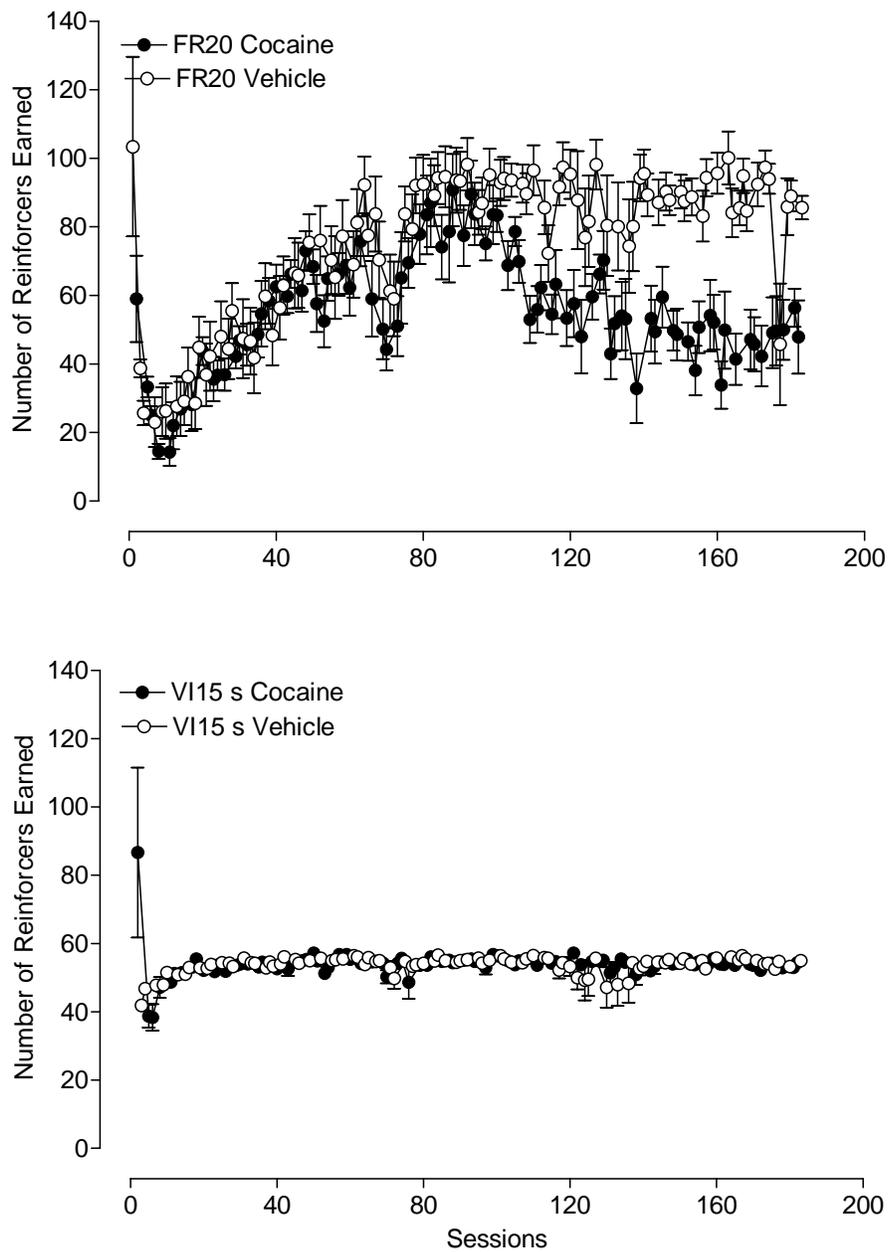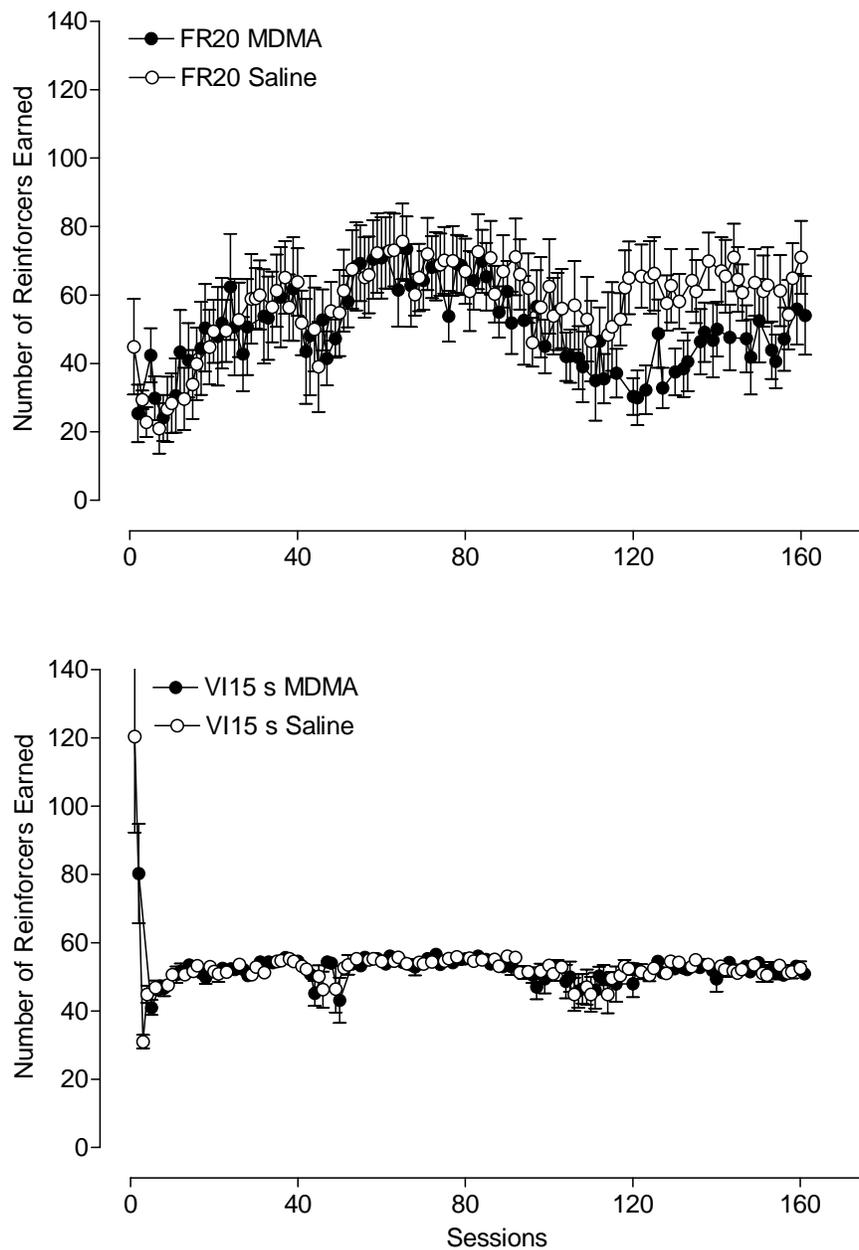
Figure 7. Frequency of reinforcement with MDMA-trained rats under drug and vehicle conditions in both FR20 (upper graph) and VI15 s schedules (lower graph). Points represent mean total number of reinforcers (n = 8 for Groups C and D). Vertical lines depict standard errors (SEM).

0.892 reinforcers under MDMA and vehicle conditions respectively. Figures 6 and 7 depict frequency of reinforcement in Groups A, B, and C, D respectively.

Marked differences in response rates and frequency of reinforcement between drug and saline conditions appeared later with rats from Groups A as shown in Figure 4 and 6. This was not the case for Groups B rats however, as response rates and frequency of reinforcement remained consistent throughout the 182 training sessions respectively. Differences in frequency of reinforcement were also found in Groups C, but not Group D. However, unlike Groups A and B, differences in response rates between drug and vehicle conditions were similar for both Groups C and D.

Stimulus Generalization Test with Cocaine

Cocaine increased the percentage of Group A rats selecting the drug-designated lever (quantal measure) in a dose-related manner as shown in the upper graph of Figure 8. Percentage of Group B rats selecting the drug-designated measure also increased in a dose-related measure. ED_{50} values obtained using the quantal measure were 2.145 mg/kg and 1.071 mg/kg for Groups A and B respectively. Unlike Group A however, percentage of Group B rats selecting the drug-designated lever exceeded the 80% substitution threshold at 1.25 mg/kg of cocaine. The large differences in percent of subjects selecting the cocaine lever between the Groups A and B gradually decreased between 2.5-10 mg/kg dose of cocaine.

With the quantitative measure, the percentage of drug-designated responses after saline administration for both Groups A and B was 32.69 ± 4.916 % (mean $\pm$ SEM). Figure 8 (lower graph) shows cocaine increase cocaine-designated responding in a dose related fashion. ED_{50} values were 1.261 mg/kg and 1.087 mg/kg for Groups

A and B respectively. The difference in drug-designated responding between Groups A and B with the quantitative measure was smaller than the quantal measure.

Stimulus Generalization Test with MDMA

Like the stimulus generalization test with cocaine, MDMA increased the percentage of Group A animals selecting the drug-designated lever (quantal measure) in a dose-related manner as shown in the upper graph of Figure 9. Percentage of Group B rats selecting the drug-appropriate measure also increased in a dose related manner. ED_{50} values were 1.400 mg/kg and 1.313 mg/kg for Groups A and B respectively. Unlike Group A however, percentage of groups rats selecting the drug-designated lever exceeded the 80% threshold at 1.25 mg/kg of MDMA. The difference in percentages of rats selecting the drug lever between Groups A and B were smaller at 2.5-10 mg/kg dose of MDMA.

Using the quantitative measure, the percentage of drug-designated lever presses after saline administration was 32.69 ± 4.916 % (mean $\pm$ SEM). The lower graph of Figure 9 shows MDMA increased cocaine-designated responding in a dose related fashion. ED_{50} values were 0.9692 mg/kg and 1.172 mg/kg for Groups A and B respectively. The difference between Groups A and B under both quantal and quantitative measures was small. There was close agreement between the two measures within each group. Disruption occurred among four cocaine-trained rats in Group A and five rats in group B. Animals that were not disrupted made more than five lever presses.
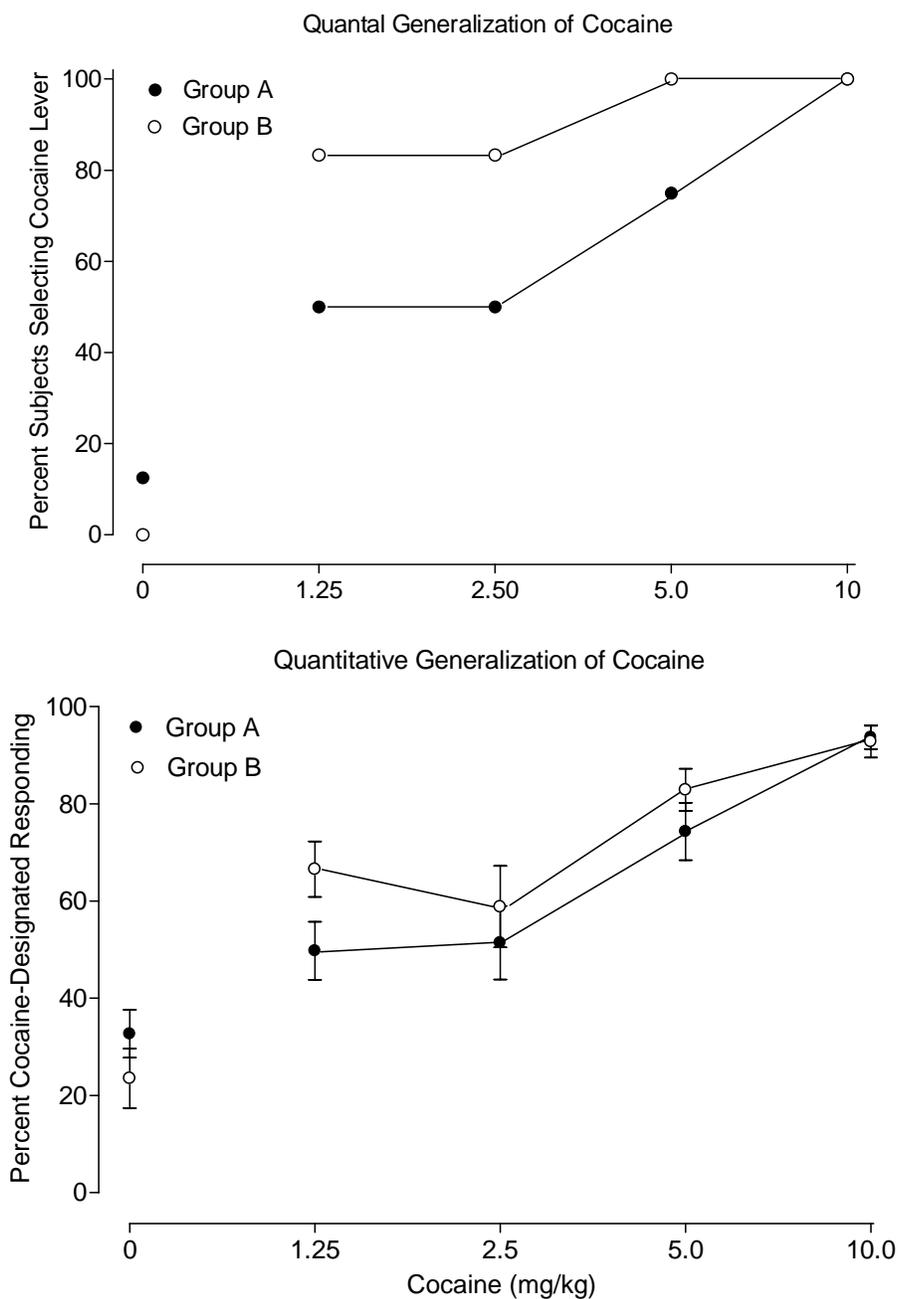
Figure 8. Cumulative cocaine dose-response functions for rats trained under an FR20 and a VI15 s schedule. Points in the upper graph represent percent subjects selecting drug lever (quantal). Points in the lower graph represent mean percent drug-designated responses (quantitative). Vertical lines indicate ± SEM.

Under the discrete dose-response procedure, MDMA also increased cocaine-designated responding in a dose related fashion in both Groups A and B. Under quantal measures, ED_{50} values were 1.035 mg/kg and 1.074 mg/kg for Groups A and B respectively. Under quantitative measures, ED_{50} values were 1.330 mg/kg and 0.9491 mg/kg for Groups A and B respectively. Unlike the previous dose-response curves that were generated using the cumulative dose-response procedure, MDMA did not substitute for cocaine at all four doses when discretely tested in Group A rats trained under the FR20 schedule. MDMA (1.5 mg/kg) did however substitute for cocaine in Group B rats trained under the VI15 s schedule (quantitative).

Three separate dose-response curves were plotted from the discrete dose-response tests using data from the first 30 s, the first 90 s, and entire 150 s session as shown in Figure 11. The dose-response curves were indexed using both and quantal and quantitative measures. From the first period of 30 seconds to the third period of 150 seconds, there is a slight increase in percent of Group A animals selecting the cocaine-designated lever and (quantal) and percent cocaine-designated responding (quantitative). For Group B however, there is a slight decrease in the percent of animals selecting the cocaine-designated lever and percent cocaine-designated responding at 0.75 mg/kg of MDMA followed by a gradual increase at 3.0 mg/kg of MDMA. Thus, for the FR group, both quantal and quantitative measures appear to be stable across all three periods and at all four doses of MDMA, whereas for Group B, both quantal and quantitative measures show a slight decrease in percentage drug designated responding at one particular dose, but was stable at all other doses.

Quantal Generalization of MDMA

Quantitative Generalization of MDMA

Figure 9. Cumulative MDMA dose-response functions for cocaine-trained rats under an FR20 and a VI15 s schedule. Points in the upper graph represent percent subjects selecting drug lever (quantal). Points in the lower graph represent percent drug-designated responses (quantitative). Vertical lines indicate ± SEM.

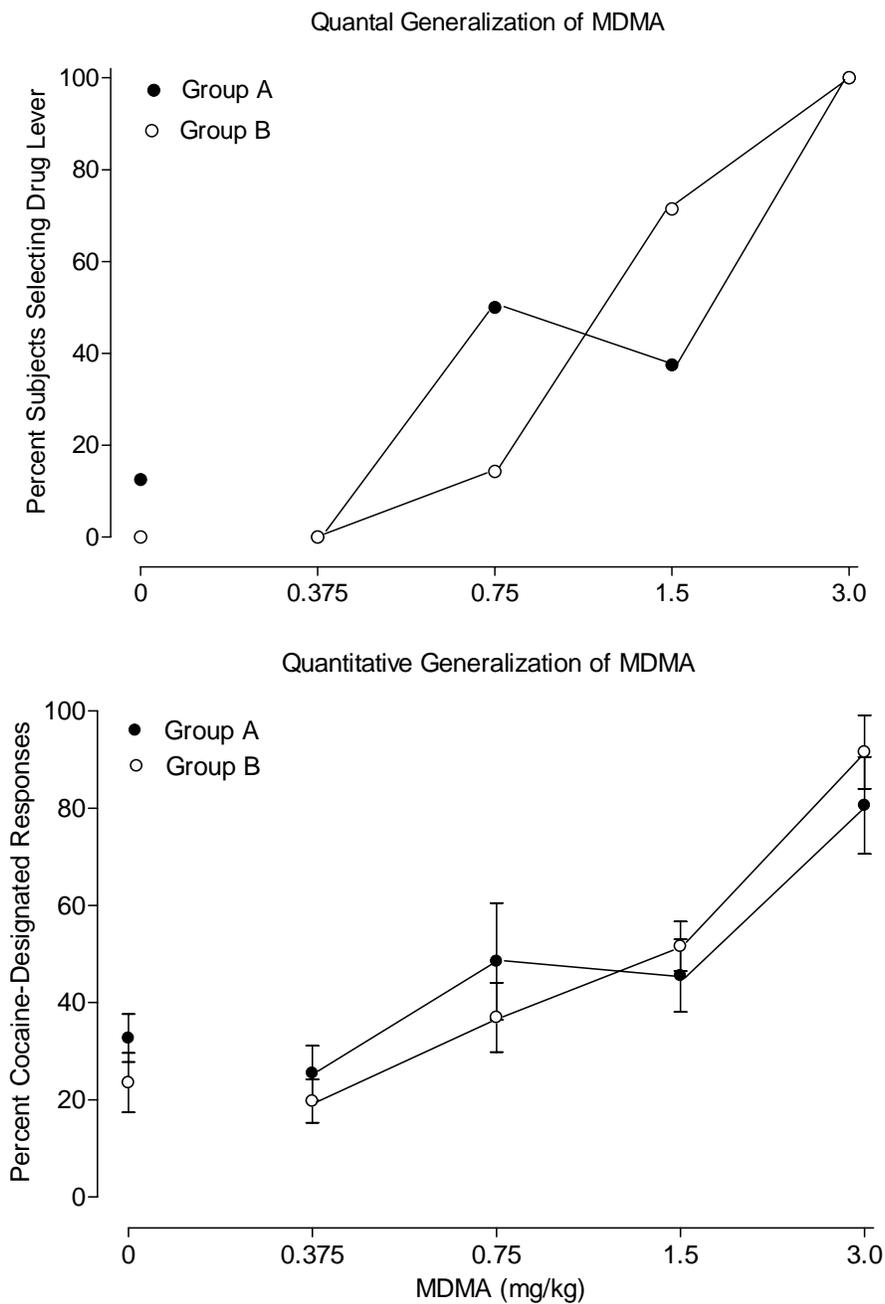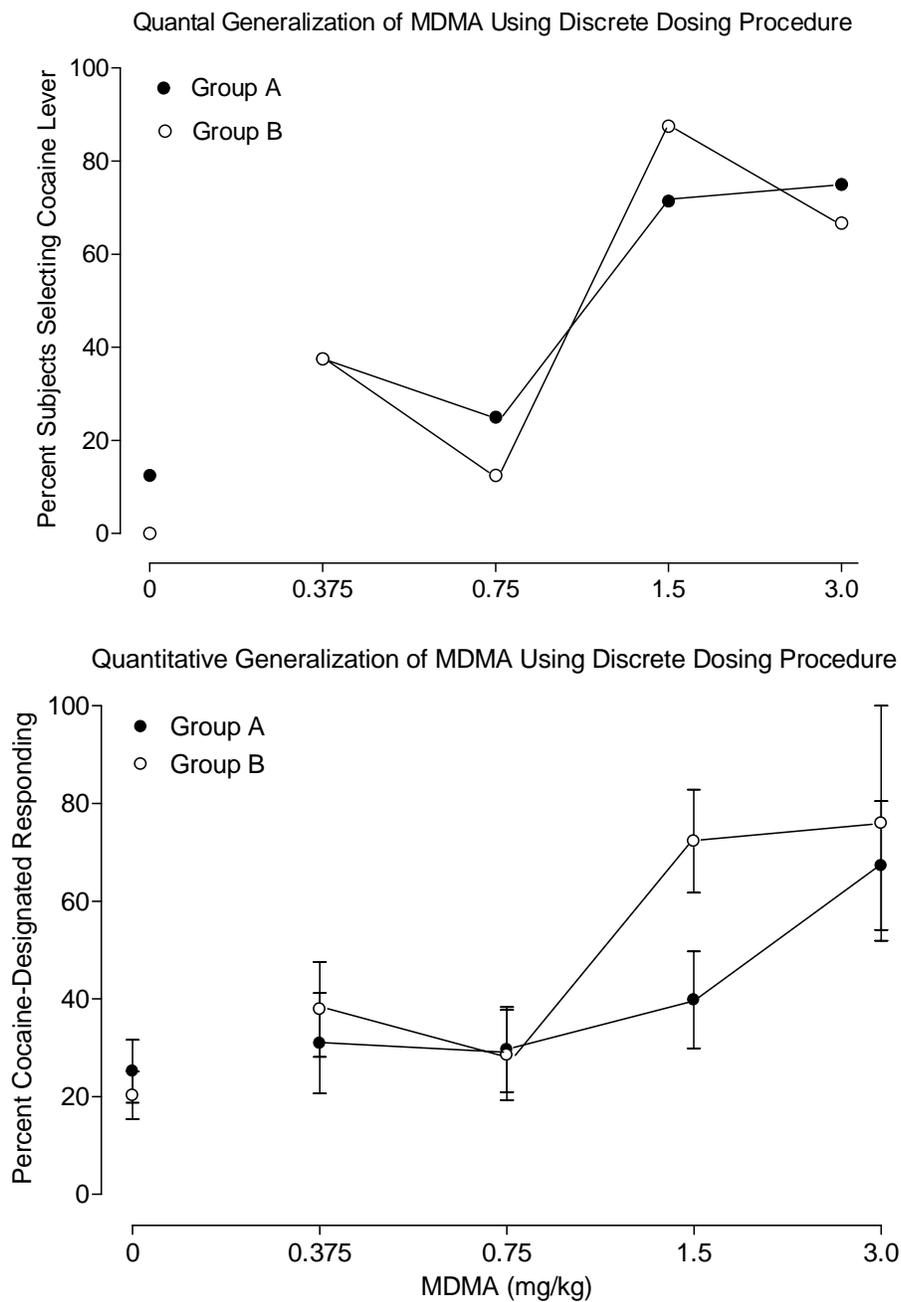Figure 10. Discrete MDMA dose-response functions for rats trained under an FR20 or a VI15 s schedule. Points in the upper graph represent percent subject selecting cocaine lever (quantal). Points in the lower graph represent percent cocaine-designated responses (quantitative). Vertical lines indicate ± SEM.
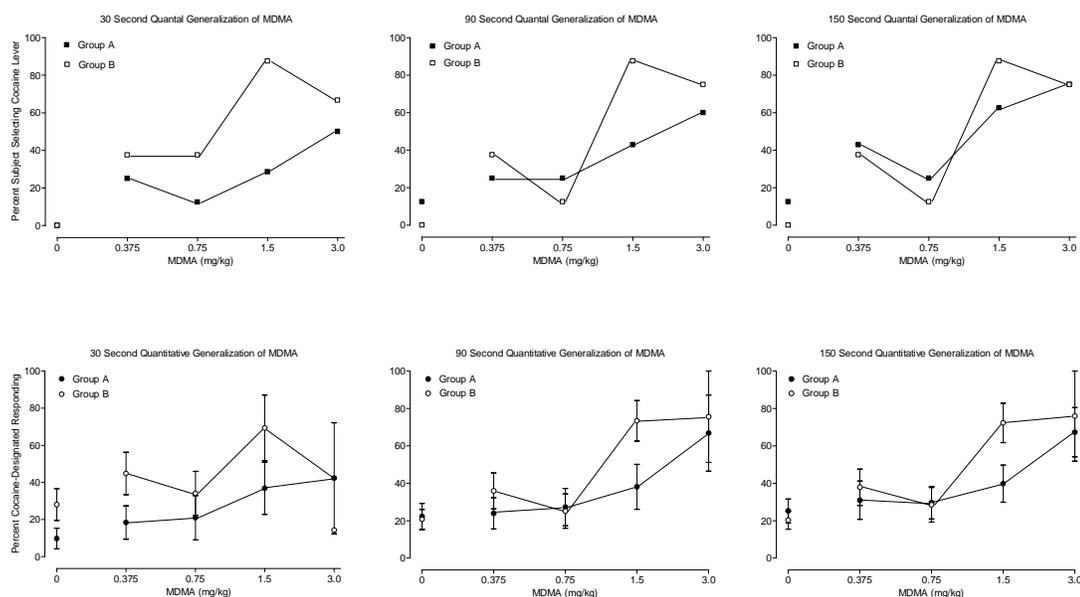
Figure 11. MDMA dose-response curves were determined from discrete dose-response procedures in cocaine-trained rats trained under the FR20 (Group A) and the VI15 s (Group B) schedule. These separate dose-response curves were plotted from the discrete dose-response tests using data from the first 30 s, the first 90 s, and entire 150 s session. Upper graphs depict quantal measures and lower graphs depict quantitative measures for three different time periods. For lower graphs, vertical lines in each bar depict standard errors (SEM).

DISCUSSION

The present study was intended to compare two commonly used reinforcement schedules in drug discrimination studies to assess the extent to which these two schedules modulate discrimination acquisition and stimulus generalization. In doing so, the results appear to be consistent with previous conclusions by other investigators. For example, the current results showed that the acquisition of cocaine and MDMA discrimination was facilitated much more rapidly under the FR20 schedule than under the VI15 s schedule. This is consistent with earlier conclusions by Overton (1979), Stolerman (1989), and McMillan et al. (2001) with other psychoactive drugs. McMillan et al. further explained that such differences were the result of the resetting component that was present in the FR schedule (McMillan, Hardwick, & Li., 2001). That is, the FR resetting component automatically reset the response counter when an incorrect lever press was made, which delayed reinforcement delivery. Thus, under the FR20 schedule, there is greater consequential control compared to the VI15 s schedule because incorrect responses are punished under the FR20 schedule, but not under the VI15 s schedule. The punishment feature of the resetting FR schedule may account for the better establishment of stimulus control by across different psychoactive drugs.

Another possible explanation for rapid drug discrimination under the FR20 schedule could be the differential patterns in response rates and frequency of reinforcement under drug and vehicle conditions that occurred with rats that were trained under the FR20 schedule. Such differential patterns in response rates and frequency of reinforcement may also help strengthen stimulus control of drugs

established by the FR20 schedule given that there were marked contrasts in stimulus conditions, response activity, and reinforcement rate under the FR20 schedule between drug and vehicle conditions, but not the VI15 s schedule (see Figures 4-7).

When using the discrete dose-response procedure, MDMA (1.5 mg/kg) only substituted in cocaine trained-rats maintained under the VI15 s schedule of reinforcement. However, dose-response curves generated using the cumulative dose-response procedure are inconsistent with previous conclusions by Khorana et al (2004) that MDMA does not substitute for cocaine in rats trained to discriminate between cocaine and saline. This was true when both quantal and quantitative indices of MDMA dose-response curves were plotted. In the Khorana et al. study, responding by cocaine-trained animals was disrupted at 1.75 mg/kg and 2.5 mg/kg of MDMA (Khorana et al., 2004). In the present study however, disruption only occurred among five of eight cocaine-trained animals, leaving only three of eight animals, each of which made > 5 lever presses. Moreover, as shown in Figure 9, MDMA (3mg/kg) also substituted for cocaine in both Groups A and B. One possible explanation for this apparent inconsistency is the difference in sample size during MDMA substitution testing. In the present study, 7-8 animals were tested for MDMA substitution whereas only 4-6 animals were tested for MDMA substitution in the Khorana et al. study (Khorana et al., 2004).

When comparing quantal and quantitative measures of stimulus generalization, similar trends in dose-response relations were found regardless of the reinforcement schedule that was used. Thus, contrary to the suggestion by Stolerman (1989), and in agreement with the conclusion by Barrett et al. (1994), the use of the

FR20 schedule does not necessarily preclude the use of quantitative measures of stimulus generalization, given that both measures yield results that are in close agreement. However, animals trained under the VI15 s made more errors than the FR20 animals, and therefore both graded and quantal measures of dose-response curves obtained from animals maintained under a VI15 s schedule should be interpreted with caution, given that there is higher variability in dose-response relations as shown in Figures 8, 9, and 10. Thus, the likelihood of obtaining false positive results may be a cause for concern.

The present results also supported the conclusion by Schechter (1997), who conducted a comparison study on dose-response curves generated using both cumulative and discrete dose-response procedures in rats trained to discriminate either cocaine or MDMA from saline. The results obtained were similar and the ED_{50} values were almost identical. However, a potential disadvantage of the cumulative dose-response procedure with animals maintained under the FR20 schedule was that response rates were lower by the time the highest drug dose was tested. Such an effect was partly due to the extinction conditions of the 2.5 minute test session and not just the disruptive effects of higher doses of MDMA. This was not the case however, for rats trained under the VI15 s schedule, given that the stimulus conditions under extinction were more similar to the conditions of VI15 s schedule but not to the conditions of the FR20 schedule.

Thus, one potential solution for controlling the effects of extinction with rats trained under the FR20 schedule is to increase the requirement of the training schedule to an FR50 schedule. A second solution would be to reduce the duration of

the test session to one minute, given that there were no sizeable differences in results

obtained from the first 30 seconds of the test session and from results obtained from

the 90 second and 150 second bins respectively (Figure 12). A third solution would

be to calculate the percentage of the first 20 responses that were made during the test

session. A fourth solution would be to reinforce drug-designated responses made

during test sessions. However, there is a conceptual problem with the fourth solution,

given that reinforcing responses made during a test session would be stating a priori

that both training and test drugs are similar. Moreover, when using a cumulative

dose-response procedure, reinforcing drug-designated responses in the first trial may

have unwanted residual effects that will bias responding in the next trial as the dose

of the test drug is progressively increased.

As stated earlier, several other variables aside from reinforcement schedules

appear to be involved in the acquisition of drug discrimination. Two other potential

variables identified thus far are number of reinforcers earned and the rate of

reinforcement, which appear to strengthen discrimination between drug and vehicle

conditions. One method for controlling the former variable would be to set a limit on

the maximum number of reinforcers that can be earned under the FR20 schedule. To

control for frequency of reinforcement, a tandem FR20-VI15s could be used to ensure

reinforcement only occurs when twenty fixed consecutive responses were made after

the passage of a variable interval. A second possible solution is to set up a system in

which occurrence of reinforcement is dependent on reinforcement that has occurred

in another chamber that was programmed for ratio schedules. Such a system is

known as *yoked boxes*, in which the chambers that are being yoked are the variable

interval chambers (Ferster & Skinner, 1957), which receives random intervals generated from rats trained under the FR schedule.

Despite the similar dose-response curves obtained using both FR20 and VI15 s schedules, it is worth considering some of the advantages and disadvantages of each schedule. There are several advantages of using the FR20 schedule over the VI15 s schedule of reinforcement. Stimulus control by a given drug is greater under the FR20 schedule compared to the VI15 s, which is ideal, given that it minimizes age-dependent factors when conducting substitution testing with other types of drugs. Moreover, as shown in Figures 8, 9, 10, dose-response curves obtained from animals trained under the FR20 schedule are more conservative compared to the VI15s which shows greater fluctuations under quantal and quantitative measures. Thus, a potential false positive is minimized when generating dose-response curves with rats that were trained under the FR schedule. Moreover, in agreement with Barrett et al. (1994), the use of FR schedules does not preclude quantitative analyses of dose-response curves as shown by the relatively small differences between these two measures in Figures 8, 9, and 10. There is also a practical incentive to using an FR schedule over a VI 15 s, since amount of time and effort spent on training subjects to discriminate drug from vehicle is significantly reduced.

However, as noted earlier, one practical advantage that the VI15 s schedule has over the FR20 schedule is that extinction effects are minimized. Moreover, in agreement with Stolerman, "probing responses" were found to be less prominent among rats trained under the VI15 s schedule (Stolerman, 1989) than with rats maintained under the FR20 schedule. Thus, when choosing a particular training

schedule in drug discrimination, it is important to consider stimulus control, time, effort, cost, and other practical constraints that are inherent in each schedule as well as the type of test session that is to be coupled to them.

CONCLUSION

The results showed that the FR20 schedule established greater stimulus control of MDMA and cocaine than the VI15 s schedule, which is consistent with previous studies showing that different reinforcement schedules can modulate the discriminative with properties of psychoactive drugs (Overton, 1979; Snodgrass, & McMillan, 1991; Stolerman, 1989). Other variables such as reinforcement frequency and response rates may also play a minor role in the acquisition of MDMA and cocaine discrimination. However, further research is needed to evaluate the extent to which these two other potential variables may be involved modulating the discriminative stimulus properties of psychoactive drugs. With respect to dose-response curves, the results are not entirely consistent with previous conclusions by other investigators that both discrete and cumulative dose-response regimens yield similar results. MDMA (ED_{50} = 0.75 mg/kg) was found to substitute for cocaine in rats trained to discriminate cocaine from saline. The use of the FR20 schedule or the VI15 s schedule does not preclude the use of either quantal or quantitative indices of dose-response relations. Despite some of the disadvantages inherent in both schedules, FR20 schedules appear to have more advantages than VI15 s schedules with respect to establishing stimulus control of a drug, minimizing training time, and interpreting dose-response curves using quantitative measures.

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APPENDIX A

The Institutional Animal Care and Use Committee (IACUC) approval form is on file

at The Graduate College, Western Michigan University.