To the Graduate Council:

I am submitting herewith a dissertation written by Elizabeth Dozier Jones entitled "Investigation of the Interaction of d-Amphetamine and Amantadine Hydrochloride Using Repeated Acquisitions with Serial Reversal Discriminations." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Psychology.

William H. Calhoun, Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:

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INVESTIGATION OF THE INTERACTION OF d-AMPHETAMINE AND AMANTADINE HYDROCHLORIDE USING REPEATED ACQUISITIONS WITH SERIAL REVERSAL DISCRIMINATIONS

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To Michael

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ABSTRACT

The purpose of this series of studies was to investigate the interaction of d-amphetamine and amantadine hydrochloride employing serial discrimination reversals with repeated acquisitions. Specifically, the blockade of the effects of d-amphetamine by amantadine hydrochloride was investigated.

In meeting this goal, dose-response curves were determined independently for d-amphetamine and amantadine hydrochloride. The dose-response curve for d-amphetamine indicated that the 0.5 mg/kg dosage provided for optimal discrimination of SDR, while dosages of 1.0 mg/kg and 2.0 mg/kg proved to be disruptive. The experiments investigating amantadine hydrochloride indicated that dosages above 10 mg/kg were detrimental to the discrimination task, while dosages below 10 mg/kg seemed to have negligible effect.

The analysis of the final experiment, in which amantadine hydrochloride was given as a pretreatment 90 minutes before d-amphetamine treatment, indicated that amantadine hydrochloride did not block the effects of d-amphetamine on learning. Rather, the interaction of the two drugs appears to be synergistic in nature rather than inhibitory.

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CHAPTER I

INTRODUCTION

Measuring Drug Effects on Learning

Research on drug effects can be loosely classified in two categories: (1) the study of drug effects on behavior (performance) and (2) the study of drug effects on learning. Generally, the first type of research is well-developed and suitable research methods exist. For example, operant schedules of reinforcement have been used in investigating the effects of drugs on behavior.

Operant schedules of reinforcement provide for good behavioral control as well as permitting cross-situational analysis of a given drug dosage under the various schedules. When utilized in this manner, the effect of a given drug dosage on such performance variables as response rate can be determined under several conditions. In addition, operant schedules provide for a repeated testing situation, or one in which each subject can serve as his own control (Boren, 1966; Kelleher and Morse, 1968; Thompson and Schuster, 1968).

In contrast to the methods used in studying the effects of drugs on performance, the methods used in studying effects of drugs on learning are not well-developed and are often insensitive. Included among the methods which have been used for this purpose are two-way shuttle box avoidance, one-trial passive avoidance, and maze learning.

Research with scopolamine (Oliverio, 1967) indicated that the drug facilitated learning in the two-way avoidance situation. However, later research using passive avoidance (Calhoun, 1971) indicated that scopolamine interfered with learning. It appeared that the drug was producing contradictory effects in two "learning" situations. In fact, the drug was causing an increase in activity which was facilitatory in the first situation and detrimental in the second. Thus, the measures used were not suitable as models for the learning process.

Similar problems are encountered with maze learning. For example, if running time were the main dependent measure in a given maze task, a drug which increased activity might result in faster maze completion, and to the false conclusion that learning was facilitated. In addition, in each of these cases it is impossible to reverse the conditions or to test the drug effect more than once on a problem. Therefore, it is impossible to note any permanent or long term changes which might result from drug usage.

From problems such as these, it is clear that there is a need for the development of an experimental methodology which would provide a sensitive measure of learning, and not solely performance. It is suggested here that the use of a repeated acquisition method similar to that employed by Boren and Devine (1968) may solve some of the problems previously encountered.

Repeated Acquisitions as a Method for Studying Drug Effects on Learning

With the repeated acquisition procedure, an animal learns a series of similar problems until acquisition on successive problems becomes

stable. Rhesus monkeys, serving as subjects in the Boren and Devine (1968) study, were required to learn a new four-response chain within each session. "A stable pattern of learning resulted, and the number of errors reached a steady state from session to session" (Boren and Devine, 1968). This steady state could then be utilized as a baseline, as suggested by Sidman (1960).

A steady state, as defined by Sidman, refers to a behavior which does not change considerably over time, but which remains stable. Therefore, any changes which take place in the steady state behavior can be attributed to the experimental manipulations. For studying drug effects, a baseline condition has several advantages. It is superior to other comparison measures in that it is reversible. That is, once the experimental condition is introduced, the behavior can return to its original state. This allows for repeated manipulations of variables in each subject, as well as for each subject serving as his own control.

On the premise that the Boren and Devine study provided a steady state of repeated acquisition, Thompson (1973; 1975) modified the design for use with pigeons. In the Thompson study, each subject was required to learn a new four-response chain daily. The task of the pigeon was to peck the correct key in the presence of one of four colors. There were three keys present in the apparatus, each capable of being illuminated by any of four colors. On any one trial, all three keys were lighted with the same color cue. Each color was correlated with one key position (left, right, or center) on a day-to-day basis. That is, on day one the sequence might be as follows:

yellow lights = peck key on right (R)
red lights = peck key in center (C)
green lights = peck key on left (L)
white lights = peck key in center (C)

The task of the pigeon was to peck the correct key for each group of colors. The same pecking sequence (R-C-L-C) was continued throughout the daily session. During the next session the sequence would be changed.

After extended training, each subject reached a stable baseline in terms of errors per session. This steady state of repeated acquisition was then used as the baseline for studying the effects of drugs on learning.

Used in this manner, with each subject serving as its own control and providing a stable daily index of learning, the repeated acquisition method appears superior to others. It controls for individual differences in learning and, due to the reversible nature of the method, can be used for repeated experimental manipulations over an extended period of time.

Learning Set as a Repeated Acquisition Method

A well documented example of repeated acquisitions is seen with the two object discrimination learning set procedure used by Harlow (1949). He found that the early performance of monkeys in a learning set situation is different from performance later in training. The Wisconsin General Test Apparatus (WGTA) was used for training monkeys in a series of two-object discriminations. The task of the monkeys was to choose the object which covered the filled foodwell.

Choice of the correct stimulus was rewarded, while choice of the other stimulus produced no reward. The position of the correct stimulus was shifted from trial to trial in a balanced order. It was found that early in training the monkeys responded in a trial-and-error manner; however, after exposure to many similar problems the probability that a monkey will make the correct choice on Trial 2 of a problem approaches 100%. It is Harlow's opinion that this change in behavior represents the development of a learning set.

Serial Discrimination Reversal Learning as a Learning Set Procedure

Just as learning set is a special case of the repeated acquisition method, so is the serial discrimination reversal learning (SDRL) method a special case of the learning set procedure. Like the learning set method, SDRL presents a complex problem to the organism and provides a method which is capable of differentiating among species according to the phylogenetic level.

The characteristics of SDRL showing its relation to learning set procedures is the change in behavior with experience. On early discriminations the animals behave in a trial-and-error fashion, but after a series of reversals learning is quite rapid and with easy discriminations animals can learn to reverse with only one error (Dufort, Guttman, and Kimble, 1954). A second characteristic of SDRL showing its relation to learning set procedures is that SDRL ranks closely related species in the same general order that learning set does (Gossette, Gossette, and Riddell, 1966) and SDRL has been suggested

as a valid procedure for the study of comparative learning ability (Gossette, 1966, 1970; Mackintosh, 1969, Sheldon, 1965).

In SDRL animals are trained to discriminate between two stimuli. When the first discrimination has been learned, the reward contingency is reversed. Training then continues through a number of such reversals. The usual measure of performance is errors per reversal and errors decrease with successive reversals. The error rate eventually reaches a stable baseline of performance which can then be used in the evaluation of various treatments.

Both simultaneous (two stimuli and two responses) and successive discrimination (one stimulus, one response) training have been used in SDRL. Most studies using rats as subjects have employed the simultaneous method, and in most cases progressive improvement has been observed in the course of the experiment (Gonzales, Berger, and Bitterman, 1966; Stretch, McGonigle, and Rodger, 1963; Dufort, Guttman, and Kimble, 1954; Mackintosh, McGonigle, Holgate, and Vanderver, 1968). Although the majority of studies have employed simultaneous SDRL there have been several of the successive type. North (1962) tested rats in a straight alley maze and did not find improvement over reversals. Clark (1967) was not able to find improvement in pigeons, but further work by Beale (1970) and Woodard, Schoel and Bitterman (1971) has established that pigeons can acquire the serial reversal strategy using successive discriminations. Beale's procedure involved a multiple variable interval schedule during S^+ and extinction during S^-. The Woodard et al. procedure involved a fixed ratio schedule during S^+ and extinction during S^-.

The best evidence of improvement in rats trained under the successive discrimination reversal method was reported by Khavari and Heise (1967). They employed a discrete trial operant procedure in which the S^+ and S^- were presented successively. Kulig and Calhoun (1972) further refined the method of Khavari and Heise. Instead of using compound stimuli like that employed by Khavari and Heise (blinking light plus tone or constant light plus tone), Kulig and Calhoun further differentiated the two stimuli by using only a light or only a tone as the S^+ or S^-.

The methodology developed by Khavari and Heise was tested for its effectiveness in drug studies by Kulig and Calhoun in its modified form. Methamphetamine was administered to rats trained on successive discrimination reversals. A change in the steady state of acquisition resulted from the administration of the drug (that is, error rate decreased), suggesting that the successive discrimination reversal procedure was sensitive to the treatment.

Justification of Successive Discrimination Reversals as a Repeated Acquisition Method

Repeated acquisition methods involve repeatedly testing animals on similar problems in order to develop a baseline for acquisition. As previously mentioned, learning set methods have been studied with a variety of species. Variations of the method exist such as Harlow (1949), Boren and Devine (1968), Beale (1970), Woodard et al. (1971), and Thompson (1973). Included in the above, and the one most likely to serve well in drug screening is the method developed by Khavari and

Heise (1967). Used in this way, successive discrimination reversal learning has the advantage, like the repeated acquisition procedure of establishing a stable baseline for each animal against which experimental treatments can be evaluated.

The successive discrimination reversal method (SDR) tests an animal first on one discrimination, and then on successive reversals of that discrimination. For example, with a tone versus light discrimination, the first S^+ (the correct and rewarded response) might be tone, with light the S^- (the incorrect and unrewarded response). During the next discrimination, the S^+ and S^- would be reversed, and so on through a series of discrimination reversals. After several sessions of successive discrimination reversals, a steady state of repeated acquisition develops. Intrasession performance represents the acquisition of any one discrimination, while intersession performance represents the repeated acquisition baseline.

Justification of SDR as a valid repeated acquisition method rests upon two empirical facts: (1) animals trained on a series of discrimination reversals develop a steady state of responding; and (2) within any one discrimination reversal errors decline directly as a function of trials. The data shown in Figure 1 were obtained from a single rat for three sessions of baseline SDR. The rat was trained on successive discrimination reversals until his performance was stable and uniform from session to session. Each session consisted of 200 trials, of which 100 were correct (S^+) and 100 incorrect (S^-). If the animal responded during a correct trial, it was rewarded. If the animal responded during an incorrect trial, the response was

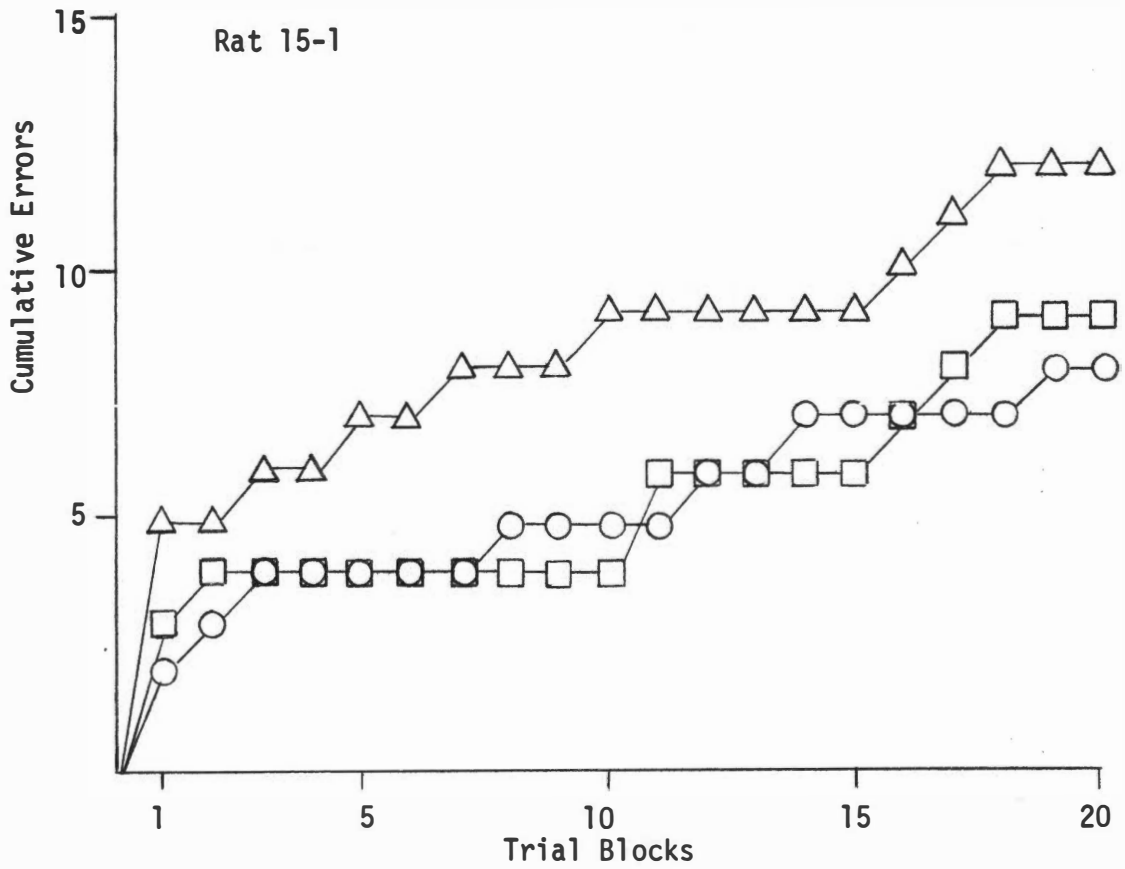


Figure 1. Cumulative error curves per 10-trial blocks for one rat over three successive sessions.

counted as an error and was not reinforced. The data in Figure 1 are plotted as cumulative errors as a function of trial blocks, with 10 trials per trial block. It should be noted that for the three sessions, the animal showed relatively uniform day-to-day acquisition. Most errors occurred in the early part of the session and errors decreased as training proceeded. For the animal represented in Figure 1, there was a marked increase in error frequency toward the end of the session. This was possibly due to satiation or fatigue. Figure 2 shows similar data for another rat. This animal demonstrates very uniform day-to-day acquisition, and the "learning curves" approximate the theoretical curves. Even though the animal had been repeatedly exposed to the light versus tone discrimination, he started each session by making a fairly large number of errors. As the session progressed, the number of errors decreased until discrimination performance was nearly perfect. Evidence from these figures demonstrates the reproducibility of the learning curve for a single animal. Thus, SDR can be used to develop a repeated acquisition baseline for studying drug effects.

In conclusion, it is suggested that the baseline SDR procedure holds promise as a method for analyzing the effects of drugs on acquisition. The advantage of this procedure is the establishment of the repeated acquisition baseline. The fact that the errors occur more frequently in the earlier part of the session and decrease as a function of trials validates the notion that the animal is actually learning the discrimination within each daily session.

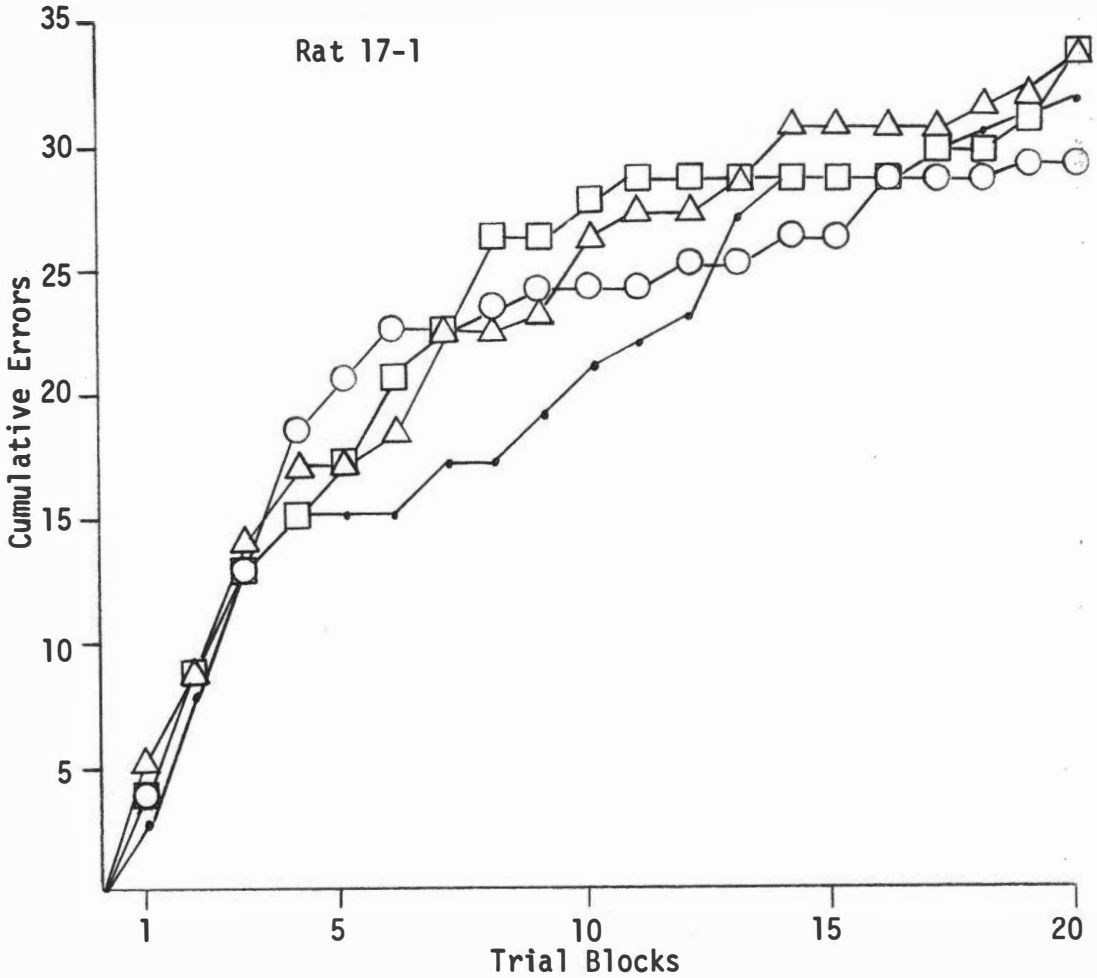


Figure 2. Cumulative error curves per 10-trial blocks for one rat over four successive sessions.

Statement of Purpose

As a group, the amphetamines have been widely investigated during the last decade. This interest has stemmed from a variety of reports, including those which claimed that this group of chemical agents increased learning ability. More recently, amantadine hydrochloride, an anti-viral agent having some stimulant properties, was reported to have blocked some of the central nervous system stimulatory effects of dextro-amphetamine.

The purposes to be served in this series of studies were threefold. The first purpose was to determine dose levels of d-amphetamine which indicate an enhancement of learning in the repeated acquisition situation. Secondly, dose levels of amantadine hydrochloride having negligible effects upon responding were to be determined. The last purpose was to evaluate the effects on acquisition when d-amphetamine and amantadine hydrochloride were given in conjunction with one another. The aim in choosing dose levels of amantadine hydrochloride which showed little or no measurable effect on acquisition was an attempt to eliminate the possibility that any effect noted in the combination drug treatment could be due to the amantadine hydrochloride alone.

CHAPTER II

THE EFFECTS OF d-AMPHETAMINE ON REPEATED ACQUISITIONS WITH SERIAL DISCRIMINATION REVERSALS

The increased popularity of the amphetamines during the past decade, along with reports that the drug caused increased alertness in the user, have led to large scale research utilizing this class of chemicals. There have also been reports of beneficial effects of amphetamine on various learning tasks. For various reasons, a great deal of the research on the effects of amphetamine on learning has used animal subjects. A review of this literature follows.

As early as 1939, the effects of amphetamine on learning were being investigated using maze learning as the task. In that year, Minkowsky tested the effects of 0.5 mg/kg dl-amphetamine on the performance of rats. The drug was administered subcutaneously 15 minutes prior to the start of the session. The results after drug administration showed an increase in errors on the maze performance. This led to the conclusion that amphetamine administration resulted in impairment of learning. Other maze studies involving the use of amphetamine followed. In 1952, Ewing, Moore, and Moore tested rats in a multiple T-maze using 1 mg/kg d-amphetamine, 8 mg/kg l-amphetamine, and saline. Early in training, a decrease in errors was observed and attributed to the amphetamine administration. However, by the seventh day of training, the error rate between groups was not significantly different. Overton (1964) reported that animals can be

trained to turn right in a simple T-maze whether or not they are drugged. Furthermore, the animals exhibit no better than chance performance while in the state in which the animal was not trained. The conclusion reached was that the drug state can serve as a discriminative stimulus in various situations.

From the maze learning paradigm interest shifted to discrimination learning. Jones (1966), Cole (1970), and Bauer and Duncan (1971) reported facilitated discrimination acquisition, performance, and reversal as a result of amphetamine treatment. Jones (1966) injected one of four doses of d-amphetamine (0.5, 1.0, 2.0, or 3.5 mg/kg) or saline intraperitoneally one hour before testing in a discrimination acquisition experiment. The first three sessions involved operant level observations of the rats in a two-lever operant chamber. For the next 10 days, the animals were reinforced whenever they pressed the lever least pressed during the three day observation period. The contingencies were reversed for the final five days of the experiment. At the 0.5, 1.0, and 2.0 mg/kg levels of d-amphetamine, the total number of correct responses increased. These dosages also facilitated the reversal. Cole (1970) found food motivated behavior to be similarly affected. He used a multiple continuous reinforcement, extinction schedule in which rats were reinforced for each lever press in the presence of light and were never reinforced in the absence of light. Under the influence of 0.5 or 1.0 mg/kg d-amphetamine, the percentage of responses during continuous reinforcement increased. The discrimination was retarded by 2.0 mg/kg d-amphetamine. All three doses increased response rate as a function of increasing dosage. Bauer and Duncan

(1971) investigated the possibility that d-amphetamine would facilitate acquisition when training was started 24 hours after the last injection of d-amphetamine. Six groups of rats were tested in an avoidance situtation (shuttle-box). The subjects received an intraperitoneal injection of either saline or 2.0 mg/kg d-amphetamine for 2, 5, or 10 days. Twenty-four hours after the last injection, the animals were placed in a shuttle-box. In their second experiment, 44 rats were tested in a Y-maze on a light/dark discrimination after receiving injections of 2.0 mg/kg d-amphetamine for either 5 or 10 days. Twenty-four hours later, they had one session of 400 trials in the maze. It was found that the multiple injections of d-amphetamine facilitated performance on both the positively and negatively reinforced tasks.

Several investigations concerned with more complex animal learning have followed. In these experiments, the organism performs most efficiently when it acquires the use of some rule such as "win stay/lose shift." Khavari (1969) administered d-amphetamine to animals which were trained according to the rule: if the previous trial was reinforced, respond; if the previous trial was not reinfoced, don't respond. On this task, d-amphetamine disrupted the performance. The number of responses on trials following an S^- response increased and the number of responses on trials one removed from an S^- response decreased. The organism appeared to be more affected by the immediate consequences of its action rather than the rule. Kulig and Calhoun (1972) reported that methamphetamine enhanced successive discrimination reversal learning. Animals were trained on a light/tone discrimination to a criterion. Upon reaching criterion, the

contingencies were reversed, and training on the reversed discrimination continued until criterion was reached. This procedure continued for eight reversals. Methamphetamine was administered 15 minutes prior to the session on Reversal 2. Doses of both 2.0 and 0.5 mg/kg reduced errors on this and later reversals. The two drugged groups maintained lower error rates than the control group animals throughout the remainder of the experiment. Finally, Calhoun and Jones (1974) reported that methamphetamine accelerated acquisition. Rats were trained through a series of light versus tone discriminations until they developed a stable repeated acquisition baseline. The animals were then injected with 0.25, 0.50, or 1.0 mg/kg methamphetamine. It was found that with low doses, 0.25 and 0.50 mg/kg, methamphetamine reduced errors without affecting rewarded responding. However, there was evidence of the drug disrupting the operant behavior at the 1.0 mg/kg level.

The majority of the data summarized here indicate that amphetamines enhance learning. However, it was felt that before trying to ascertain the interaction effect of any drug combination, it was first necessary to establish the effects of several dose levels of each drug independently. Because it is the blockade of the effect of d-amphetamine that is under investigation in this series of studies, it was desirable to determine a dose level which produced a measurable effect. The first experiment was conducted to determine the appropriate levels of d-amphetamine.

General Method

Subjects

The subjects were six experimentally naive, male Wistar rats (250-350 g) obtained from the Holtzman Co., Madison, Wisconsin. The animals were housed three per cage and were maintained on a 23 hour water deprivation schedule during the experimental testing. Food was available ad libitum throughout the course of the experiment.

Apparatus

Six identical one-lever operant chambers (Lafayette Instruments) measuring 28 x 21 x 21 cm were used. The lever, requiring 25 g for micro-switch closure, was mounted 7 cm above the floor of the chamber in the center of one wall. A 0.10 ml drop of a 9% sucrose solution was presented as reinforcement in a spout mounted 6 cm to the left of the lever. Stimuli consisted of a 7 W white cue light located 6 cm directly above the lever, and a 1000 Hz tone presented by a Sonalert located 10 cm behind the response lever. A 7 W red houselight provided constant illumination. Each operant chamber was enclosed in a sound attenuating chamber; these were contained in three 1.3 x 3.2 x 2.2 m experimental cubicles. Fans provided ventilation and masking noise. Stimuli were programmed with electromechanical circuitry located outside the experimental cubicles. Data was collected on counters.

Procedure

The subjects were first adapted to a 23 hour water deprivation schedule. For three days prior to training, the subjects were allowed

access to water for only one hour each day. Following this adaptation, the subjects were shaped to bar press for 0.1 cc reinforcement under a continuous reinforcement schedule. Neither the light nor the tone was presented during this phase of training. The subjects were trained under this schedule for one-half hour a day for three days. Following acquisition of the bar pressing response, the discrete trial procedure was begun. Daily sessions for each subject consisted of 200 trials. Either the tone or the light signalled a six-second trial, separated from the following trial by a 10-second intertrial interval (ITI). A 20 unit Gellerman series ordered presentation of the trial stimuli. A lever press during an S^+ (correct) trial terminated the trial and produced reinforcement. Lever presses during S^- (incorrect) trials had no effect; the trial stimulus continued for the programmed six seconds. The first lever press during an S^- trial was called an error. Any response during the 10 second ITI reset the interval timer, thus prolonging the ITI. Subjects were allowed free access to water for one hour following each daily session.

SDR training continued until performance stabilized, at which time administration of d-amphetamine began. All injections were intraperitoneal and were given 15 minutes prior to the session. Each animal received .001 cc of solution per gram of body weight of four doses of the drug (0.5, 0.75, 1.0, and 2.0 mg/cc) and one control injection of physiological saline. Of the many possible permutations of the five dosages, 24 were determined. From these 24 drug sequences, six were selected. One of these was assigned to each subject. In this way the order of drugging was randomized. A restabilization period followed each treatment.

Experiment I. Each of the six subjects was run on SDR until stabilized. There were two requirements of a stable baseline. First, the animal was required to maintain five day error range equal to or less than 10. In addition, all scores within the five day period had to fall within the range defined by the mean of the five days of error scores plus or minus one-half the average of those scores. At this point the subjects were injected with one of the following: isotonic saline, 0.5, 0.75, 1.0, or 2.0 mg/kg d-amphetamine. Each subject received each dose level. The order of administration of the various drug treatments was randomized for each subject. Fifteen minutes following the intraperitoneal injection the animals were run as usual.

Results

The data were analyzed in terms of S^+, S^--T, S^--I, and ITI responding. Separate analyses of variance were performed on each of the response measures. This was done in order to present a more accurate representation of the effects of d-amphetamine on SDR responding. The statistical design used to test the results was that of a single factor experiment with repeated measures.

Analysis of variance on the effects of the various drug doses on S^+ responding was significant ($F=13.04$; $df=4,20$; $p<.01$). Inspection of Table I and Figure 3 indicates that the dose levels of 0.5 and 0.75 mg/kg have little effect on S^+ responding. In contrast, administration of 1.0 mg/kg d-amphetamine resulted in a large decrease in S^+ responding. Administration of 2.0 mg/kg d-amphetamine severely

TABLE I

Mean SDR Performance as Related to d-Amphetamine Dosage

Dose	SDR Measures			
	S^+	S^--T	S^--I	ITI
Saline	99.2	12.5	9.0	5.2
0.50	99.5	5.2	3.7	7.7
0.75	99.0	9.3	5.7	3.7
1.00	72.8	4.2	2.7	5.8
2.00	17.7	0.8	0.8	2.3

disrupted responding on all SDR measures (Table I, Figure 3, Figure 4).

Although an analysis of variance did not reach significance for S^--T responding ($F=2.18$, $df=4,20$), the analysis for S^--I responding was significant ($F=3.20$; $df=4,20$; $p<.05$). Figure 4 shows the error scores for the individual animals. Five of the six animals show a decline in number of errors at the 0.5 mg/kg dose level. At 0.75 mg/kg, two animals showed a further decrease in errors. At 1.0 mg/kg, error scores appeared to decrease; however, reinforced behavior was diminished as well, indicating disruption of performance.

Inspection of Table I indicates that all dose levels of d-amphetamine tested had little effect on ITI responding. This is supported by an analysis of variance on ITI responding which failed to demonstrate significant differences between the dose levels ($F=1.13$; $df=4,20$).

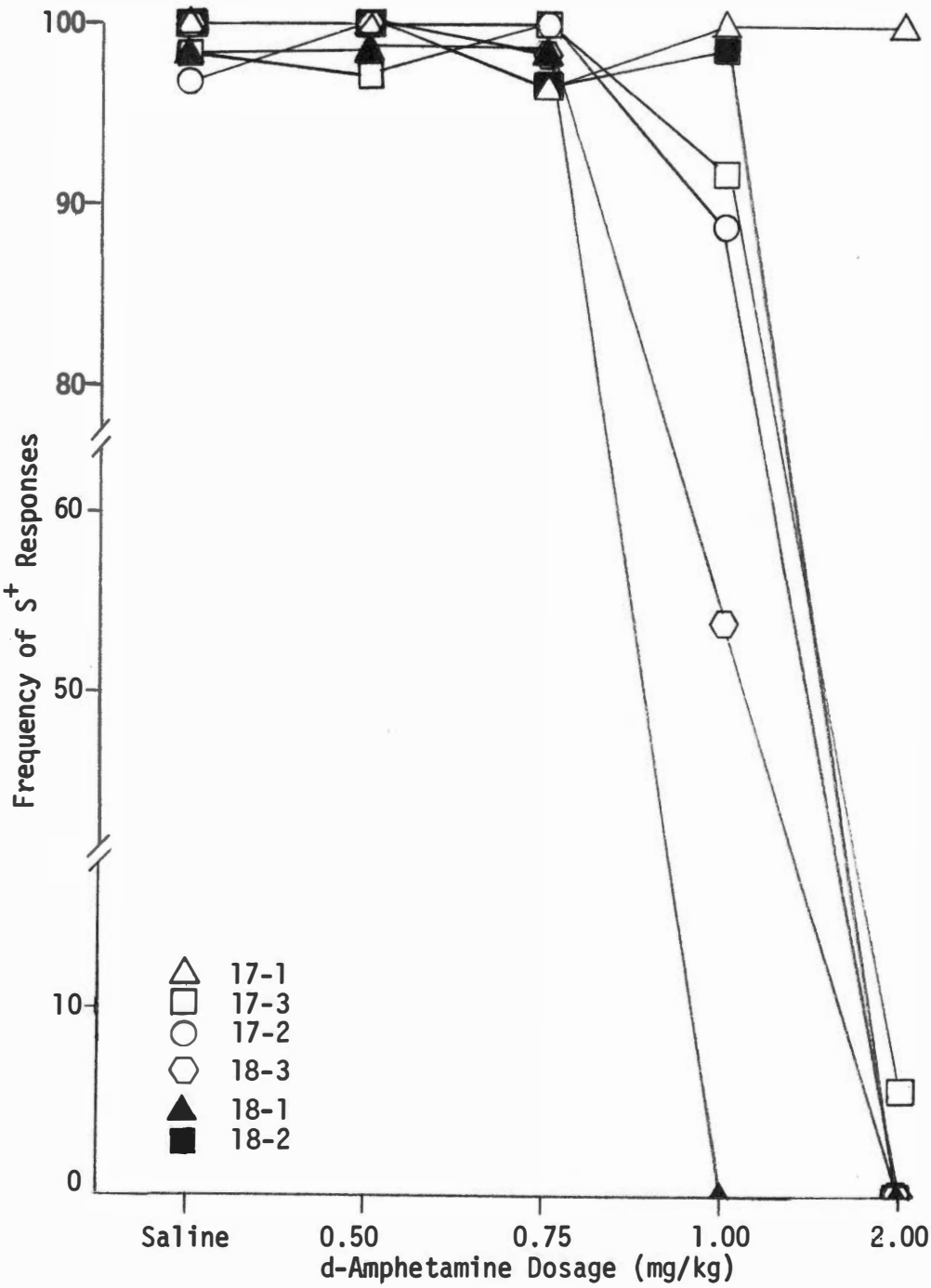


Figure 3. Number of S^+ responses under each drug condition for 6 Ss.

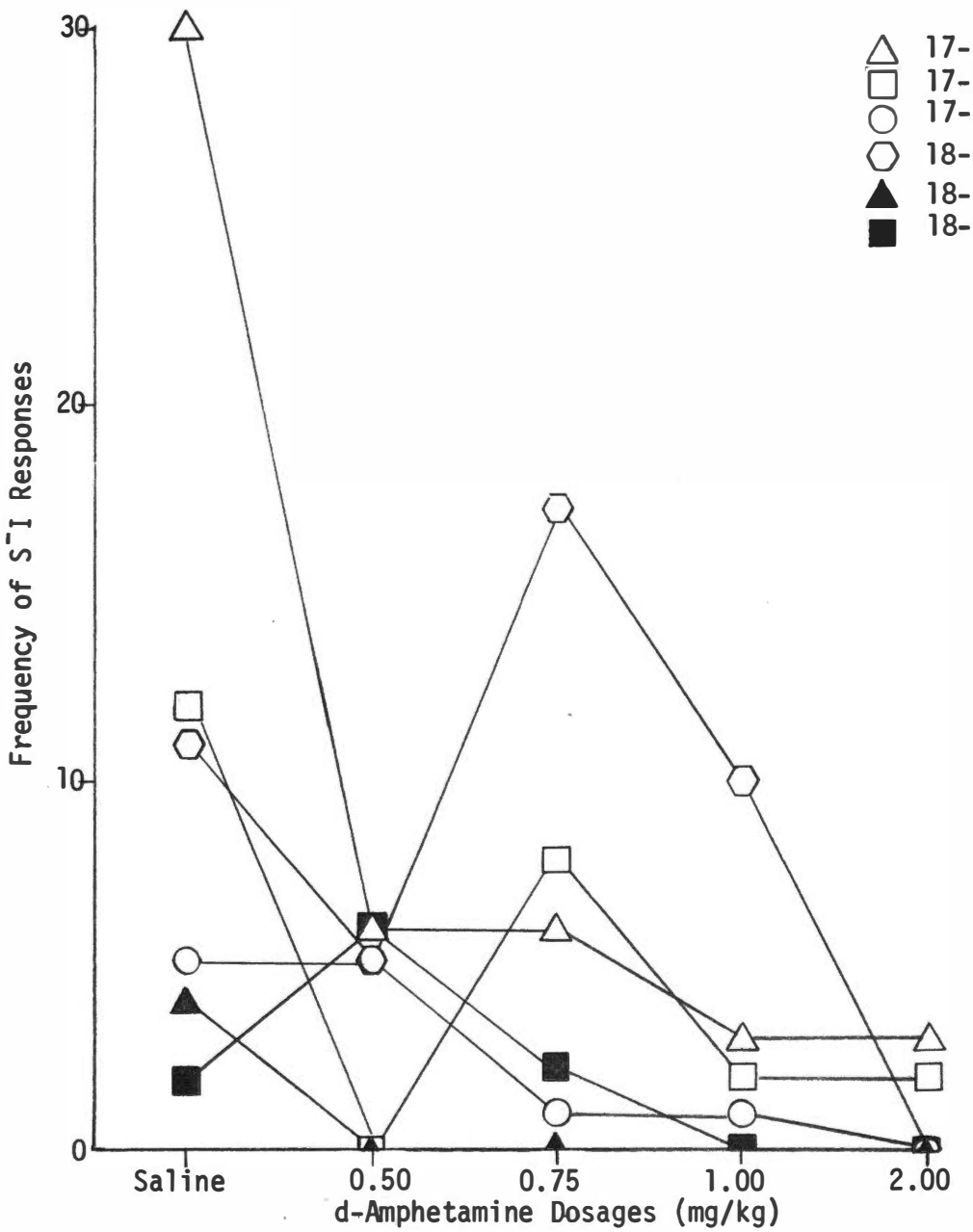


Figure 4. Number of S⁻I responses under each drug condition for 6 Ss.

Discussion

The dose levels tested appear to provide a suitable range of dose levels for the later studies of this series. The most effective dose tested for reducing errors was 0.5 mg/kg. The consistency of responding between subjects noted at the 0.5 mg/kg level diminishes at the 0.75 mg/kg and 1.0 mg/kg levels. While some animals have decreased error rates at these levels, the remaining animals appear to have experienced some disruption. This effect is not as notable at 0.75 mg/kg as at 1.0 mg/kg, implying that 0.75 mg/kg is approaching a disruptive level. Similarly, although 1.0 mg/kg is not as disruptive as 2.0 mg/kg, it is disruptive for three of six subjects. Clearly, 2.0 mg/kg results in severe disruption of all SDR responding. Because of the highly disruptive nature of this dose level, 2.0 mg/kg was eliminated as a dosage for further experimentation in studies reported here.

CHAPTER III

THE EFFECTS OF AMANTADINE HYDROCHLORIDE ON REPEATED ACQUISITION WITH SERIAL DISCRIMINATION REVERSALS

Amantadine hydrochloride (1-adamantananine hydrochloride) has been reported (Vernier et al., 1969; Strömberg and Svensson, 1971) to be a central nervous system stimulant in experimental animals. However, it was recently reported by Menon, Clark, and Fleming (1973) that the hyperactivity caused by d-amphetamine was greatly reduced in amantadine HCl pretreated mice. For this reason, amantadine hydrochloride was selected as the drug to be administered in conjunction with d-amphetamine to determine what effect it might have on the central nervous system effects on d-amphetamine on SDR responding.

Very little research on the behavioral effects of amantadine HCl has been reported. Those that have, like the Menon, Clark, and Fleming study, are mainly concerned with motor activity and gross behavioral signs of central nervous system stimulation. The majority of the studies, a few of which are reported here, are concerned with the mechanism of action of the drug.

In a series of studies reported by Vernier et al. (1969) it was reported that at high dose levels central nervous system stimulation, anorexia, emesis, and some convulsions were noted. Included in the catch-all "signs of CNS stimulation" were: increased spontaneous motor activity, antagonism of tetrabenazine-induced sedation,

piloerection, tremors, increased sensitivity to environmental stimuli, and convulsions. In the Vernier et al. report, toxic effects for at least five dose levels were given for several species. The toxic signs produced by lethal or near-lethal doses of amantadine HCl in these species were similar. Signs of CNS stimulation, followed by tremors and clonic convulsions were common to three rodent species (mice, rats, and guinea pigs) by all routes of administration. Death was usually preceded by signs of respiratory distress and convulsions. The LD-50 for female rats was reported to be 223 mg/kg when administered intraperitoneally; and the peak effect of the drug was reported to occur two hours after injection.

Vale, Espejil, and Dominguez (1971) reported a controlled trial of amantadine in depressive cases. Forty patients with two examiners' diagnoses of "chronic depressive syndrome" were used as subjects. The drugs the patients were taking were continued unchanged and each patient recieved at random one of three treatments:

Group A (10 Ss):	100 mg daily for weeks 1 and 2 200 mg daily for weeks 3 and 4
Group B (10 Ss):	200 mg daily for weeks 1 and 2 100 mg daily for weeks 3 and 4
Group C (20 Ss):	were given an inactive preparation for four weeks, with the number of pills being increased or decreased as in Groups A and B.

Thirty-four subjects completed the experiment. There were no significant differences between groups A and B; they were, therefore, combined for the rest of the evaluation. Restlessness and anxiety were apparent in 20 percent of the patients on a mean of 10 days. The difference between the combined AB group and Group C was

significant (14/18 in AB showed improvement while only 6/16 in C). The conclusion reached was that although amantadine is less effective than some other agents in the treatment of depression, it does have some antidepressant properties.

Cox and Williams (1972) investigated the cardiovascular actions of amantadine and attempted to confirm its interaction with dopamine. They concluded that amantadine is a weak pressor agent in the rat and rabbit but that is neither dopamine-like or tyramine-like in its action.

Several other studies were concerned with the mechanisms of action of amantadine. Fletcher and Redfurn (1970) concluded from the Vernier et al. report of 1969 that high doses of amantadine might inhibit uptake of noradrenalin into peripheral nerve endings. It was reasonable then to suppose a similar action would take place on the uptake of dopamine and would help explain the antiparkinsonian effects of amantadine. Results of this experiment, however, make it appear unlikely that the central stimulation of amantadine would be due to the inhibition of uptake into central nerve endings. Herblin (1972) also examined the effects of amantadine on the uptake of norepinephrine and dopamine by synaptosomes from the striatum and hypothalamus of normal and reserpine treated rats. He found that amantadine HCl inhibits the uptake of norepinephrine in both areas but is more potent against the hypothalamic system. Reserpine has little effect on either amount of uptake or the extent of inhibition. Like the Fletcher and Redfurn study, the results here make it seem unlikely that inhibition of uptake is involved.

Several studies have examined the effects of amantadine HCl on locomotor activity in the rat. Fibigar, Fox, McGeer, and McGeer (1971) found significant increase in activity which is dose and time dependent. The doses used in the study were 20, 50, or 100 mg/kg amantadine in an aqueous solution. One-half hour after a subcutaneous injection of the drug, the animal was placed in a 35 cm diameter cage. Movement was recorded for three hours. All doses produced significant increases in activity: 25 mg/kg increased activity early in the three hour period; 50 mg/kg increased activity throughout the period, and 100 mg/kg increased activity during the last hour of the three hour period. Another study investigating stimulation of motor activity by amantadine was carried out by Maj, Sominska, and Baran (1972). In this study, intraperitoneal injections ranging from 20 mg/kg to 80 mg/kg were used. It was found that amantadine moderately stimulates motor activity. Doses of 80 mg/kg doubled activity while higher doses depressed it. For the dose range investigated, amantadine abolished induced catalepsy. A third study, reported by Lassen (1973), compared amantadine with amphetamine as to its effect on motility. Drugs were administered subcutaneously and motility was measured for six hours following drug administration. Treatment with either of the two drugs increased motility significantly as compared to the saline control group. In the discussion of his findings, Lassen reports:

> The behavior effects of amantadine in rats may depend on a combination of mechanisms involving certain catecholamine systems. Amantadine may exert both a DA-receptor stimulating effect as well as a DA and NA release. The rotational behavior elicited by amantadine in rats with unilateral nigrostriatal lesions indicated that dopamine release predominates over dopamine acceptor stimulation in the striatum.

> The failure of amantadine to elicit stereotyped behavior suggests that it activates a special dopamine system or simultaneously activates a NA system with behavioral modulating effect.

The final study, also involving activity, is that of Menon, Clark, and Fleming (1973) which investigated the effects of amantadine pretreatment on d-amphetamine induced hyperactivity in mice. Within 15 minutes animals treated (i.p. injection) with either 2.0 or 5.0 mg/kg d-amphetamine showed hyperactivity. Animals receiving amantadine (150 mg/kg) alone were hyperactive immediately after injection but, unlike d-amphetamine, jerky movements occurred unaccompanied by exopthalamos and piloerection. The animals were sensitive to touch and some convulsions were noted. Pretreatment with amantadine completely blocked practically all the effects of amphetamine.

In order to ascertain whether the effects of d-amphetamine could be blocked by amantadine, it was important to determine a dosage of amantadine which did not cause a substantial effect in its own right, but one that merely blocked the effect of amphetamine usually observed.

The lower limit of LD-50 for amantadine in female rats was reported to be 167 mg/kg (Vernier et al., 1969). However, as low a dose as 100 mg/kg reportedly caused a significant decrease in weight gain as well as resulting in increased mortality. Also, behavioral signs of CNS stimulation were evident at this dosage. In the studies reported by Vernier et al., the lowest dose used was 16 mg/kg which was virtually ineffective.

Because of these reports, Experiment II was undertaken to

investigate the effects of several dosages of amantadine in the range of 10 mg/kg to 100 mg/kg. It was hoped that this would provide an upper and lower limit for the effects of the drug for the purposes of this study. When the data from Experiment II indicated the desirability of using a lower dose level, Experiment III was performed to ascertain the effects of three lower dosage levels of amantadine hydrochloride on SDR performance.

General Method

Subjects

Experiments II and III each employed 12 experimentally naive, male Wistar rats (250-350 g) obtained from Holtzman Co., Madison, Wisconsin. The animals were housed three per cage and were maintained on a 23 hour water deprivation schedule during the experimental testing. Food was available ad libitum throughout the course of the experiment.

Apparatus

The apparatus used in Experiment I was utilized.

Procedure

The procedure was identical to that used in Experiment I.

Experiment II. The six subjects were run on SDR until performance stabilized. The same criterion for stability as in Experiment I was used. After reaching a stable baseline, the subjects were injected with one of the following: isotonic saline, 10, 20, 40, or 80 mg/kg amantadine hydrochloride. Each subject received each dose

level. The order of administration of treatments was randomized for each subject. Injections were intraperitoneal and were given 90 minutes prior to the 200 trial session.

Experiment III. Six naive subjects were run on SDR until performance stabilized. At this point each subject received one of the following: isotonic saline, 2.5, 5.0, or 7.5 mg/kg amantadine hydrochloride. Each subject received each dose level, with order of administration randomized for each subject. Injections were intraperitoneal and were given 90 minutes prior to the session.

Results

The data for Experiments II and III were analyzed in terms of the four response measures. In each experiment an analysis of variance was performed on each of the four SDR measures: S^+, S^--T, S^--I, ITI. A single-factor, repeated measures design was employed in the analysis. In Experiment II, the analyses of variance performed on S^+, S^--T, and S^--I were significant (S^+: $F=18.15$, $df=4,20$, $p<.01$; S^--T: $F=19.61$, $df=4,20$, $p<.01$; S^--I: $F=17.57$, $df=4,20$; $p<.01$). There was little effect on ITI responding ($F=2.46$, $df=4,20$). Inspection of Table II indicates that increasing dosage of amantadine, 10, 20, 40, and 80 mg/kg reduced responding on S^+. The drug initially elevated errors as indicated by the means for both S^--T and S^--I. ITI responding was little effected except at 80 mg/kg where the animals were generally debilitated and performed very little if at all.

The results of Experiment III were analyzed in the same manner as those of Experiment II. No significant differences were found.

TABLE II

Mean SDR Performance as Related to Amantadine Hydrochloride--High Dosages

Dose	SDR Measures			
	S^+	S^--T	S^--I	ITI
saline	95.3	27.5	16.5	16.8
10.0	95.3	42.0	40.0	12.5
20.0	87.5	69.2	42.3	13.2
40.0	70.7	52.3	35.3	17.0
80.0	15.5	19.8	13.5	2.2

Inspection of Table III supports this statistical evidence. As dosage increased from 2.5 to 7.5 mg/kg, S^+ responding declined very little. Likewise, S^--T, S^--I, and ITI responding showed little, if any, change.

Discussion

For the purpose of this series of studies, the dosages tested in Experiment II were clearly too high. The highest dosage tested, 80 mg/kg, resulted not only in severe disruption of performance, but also in symptoms of CNS over-stimulation. Several animals were observed to have convulsions at this dose level.

The effects of the other dosages tested in Experiment II were not as severe as the 80 mg/kg dose level; however, all caused a decline in performance. These results indicated that a downward extension of the dosage levels was needed.

Experiment III, testing 2.5, 5.0, and 7.5 mg/kg amantadine, provided the level of dosage needed. At the lowest dose, little effect

TABLE III

Mean SDR Performance as Related to Amantadine Hydrochloride--Low Dosages

Dose	SDR Measures			
	S^+	S^--T	S^--I	ITI
saline	99.5	16.7	10.5	13.7
2.5	99.2	19.5	13.3	11.2
5.0	98.0	22.0	15.7	15.0
7.5	93.5	19.2	13.0	11.8

on any SDR measure was observed. Effects after injections with 5.0 and 7.5 mg/kg were also negligible. From these results, all dosages in Experiment II were eliminated for further use in this study. Because there was so little difference in the dosages tested in Experiment III, it was determined that all three, 2.5, 5.0, and 7.5 mg/kg, would be used in the final study in this series.

CHAPTER IV

AN INVESTIGATION OF THE INTERACTION OF d-AMPHETAMINE AND AMANTADINE HYDROCHLORIDE USING REPEATED ACQUISITIONS WITH SERIAL REVERSAL DISCRIMINATIONS

It it known that the central stimulatory effect of d-amphetamine is correlated with its ability to cause an increased release of brain dopamine. Because of this, the pharmacological effects of d-amphetamine are effectively blocked either by drugs which block the central dopamine receptors or by those which block the synthesis of dopamine. These amphetamine antagonists are central nervous system depressants. The interesting aspect of the Menon, Clark, and Fleming (1973) study is that amantadine, a central nervous system stimulant which has effects in common with those of d-amphetamine, effectively blocked the pharmacological and biochemical effects of d-amphetamine.

Several reasons for this effect have been suggested, the most popular of which is competitive antagonism. Drugs acting at the same site are capable of blocking the effects of one another. A similar site of action also means that the agonist and antagonist will have similar pharmacological effects. Many similarities between the actions of amantadine HCl and d-amphetamine could be cited. As well as the central nervous system stimulant properties such as enhanced motor activity (Vernier et al., 1969; Strömberg and Svensson, 1971), both cause unilaterally striatectomized rats to rotate toward

the operated side (Strömberg and Svensson, 1971), and cause inhibition of uptake of catecholamines by central nerve terminals (Coyle and Snyder, 1969; Scatton et al., 1970). The central effects of both drugs are inhibited by alpha-methyltyrosine (Strömberg et al., 1970) and both drugs cause a decrease in the accumulation of brain norepinephrine after l-dopa (Strömberg et al., 1970). From these similarities it is reasonable to suggest that these drugs act at the same site, and due to its resistence to biotransformation in mice (Bleidner et al., 1965) it is further suggested that amantadine stays at the site of action a long time, thereby preventing amphetamine from exerting its usual effects.

Experiments I, II, and III determined the dose levels for d-amphetamine and amantadine hydrochloride which provided the appropriate effects for use in Experiment IV. In Experiment IV, combinations of the various dose levels of each drug were administered. The purpose of the experiment was to determine whether amantadine hydrochloride could block the effects of d-amphetamine usually observed on SDR performance.

General Method

Subjects

The subjects were 24 experimentally naive, male Wistar rats (220-250 g) obtained from Holtzman Co., Madison, Wisconsin. The animals were housed three per cage and were maintained on a 23 hour water deprivation schedule during the experimental testing. Food was available ad libitum throughout the course of the experiment.

Apparatus

The apparatus described in Experiment I was used in the present study.

Procedure

The discrete trial operant SDR procedure described in Experiment I was used in the present study.

Experiment IV. The 24 animals were randomly divided into four groups of six animals. After reaching a stable baseline, each animal received, 90 minutes prior to the session, one of the following: isotonic saline, 2.5, 5.0, or 7.5 mg/kg amantadine hydrochloride. Fifteen minutes prior to the session, the animals were injected with the dosage of d-amphetamine assigned to its group: 0.5, 0.75, or 1.0 mg/kg, or saline. The order of amantadine treatments within each group was determined as in previous experiments. All injections were intraperitoneal.

Results and Discussion

The statistical design used to test the results was a two-factor analysis of variance with repeated measures on one factor. A separate analysis of variance was performed on each of the dependent measures: S^+, S^--T, S^--I, and ITI.

The analyses for S^+ responding (Table IV) and ITI responding (Table V) indicated that there were no significant differences between the dose levels of amantadine. Similarly, these analyses showed non-significant effects due to the interaction between amantadine and

TABLE IV

Summary Table for Analysis of Variance:
S^+ Responding

Source	df	SS	MS	F
Between Subjects	23	10,070.12		
Amphetamine dose levels	3	1,094.21	364.70	0.81
Error between	20	8,975.92	448.80	
Within Subjects	72	11,772.50		
Amantadine dose levels	3	1,134.54	378.20	2.56
Interaction	9	1,700.10	188.90	1.28
Error within	60	8,877.85	147.96	
Total	95			

TABLE V

Summary Table for Analysis of Variance:
ITI Responding

Source	df	SS	MS	F
Between Subjects	23	27,730.33		
Amphetamine dose levels	3	2,759.41	919.80	0.74
Error between	20	24,970.90	1,248.55	
Within Subjects	72	32,007.00		
Amantadine dose levels	3	1,994.41	664.80	1.46
Interaction	9	2,711.18	301.24	0.66
Error within	60	27,301.41	455.02	
Total	95			

d-amphetamine. These results for drug combinations on S^+ and ITI responding were not surprising. Because the animals are usually performing at close to optimal levels on S^+ trials it is difficult for results following drug administrations to reach significance unless a large decrement in S^+ responding takes place. ITI responding is often little affected by drug trials. This observation was upheld by the data obtained in Experiment IV which showed no significant differences in ITI responding resulting from various amantadine dosages, d-amphetamine dosages, or from an interaction of the two drugs.

The analyses of the S^--T and S^--I data revealed results similar to those for S^+ and ITI responding. For both measures, the effects due to amantadine dose levels, or to the interaction between d-amphetamine and amantadine dose levels, were non-significant; however, the effects due to the various d-amphetamine dose levels was significant (see Table VI and Table VII). Further analyses of the

TABLE VI

Summary Table for Analysis of Variance:
S^--T Responding

Source	df	SS	MS	F
Between Subjects	23	11,840.24		
Amphetamine dose levels	3	6,097.20	2,032.40	7.08*
Error between	20	5,743.04	287.15	
Within Subjects	72	9,822.25		
Amantadine dose levels	3	814.78	217.59	1.65
Interaction	9	1,115.34	123.93	0.94
Error within	60	7,892.13	131.54	
Total	95			

*$p<.01$

TABLE VII

Summary Table for Analysis of Variance:
S^--I Responding

Source	df	SS	MS	F
Between Subjects	23	5,378.00		
Amphetamine dose levels	3	3,200.33	1,066.78	9.80*
Error between	20	2,177.67	108.88	
Within Subjects	72	2,966.50		
Amantadine dose levels	3	218.58	72.86	1.74
Interaction	9	243.92	27.10	0.65
Error within	60	2,505.00	41.75	
Total	95			

*$p<.01$

S^--T and S^--I data indicated there were no significant differences between the d-amphetamine groups following pretreatment with 5.0 mg/kg amantadine (see Table VIII and Table IX). However, pretreatment with saline, 2.5, or 7.5 mg/kg amantadine did result in significant differences between the d-amphetamine dose levels (see Tables X to XV). Tukey-hsd multiple comparison tests were performed on each of these significant analyses.

The results of the multiple comparison tests were essentially the same for S^--T and S^--I responding. Following pretreatment with either saline or 7.5 mg/kg amantadine there were significant differences between the control treatment (saline) and all dosages of d-amphetamine ($p<.05$). However, there were no significant differences between the various d-amphetamine dose levels. Following pretreatment

TABLE VIII

ANOVA Summary Table: S^--I Responses. Differences in d-Amphetamine Dosages with 5.0 mg/kg Amantadine Pretreatment

Source	df	SS	MS	F
Amphetamine dose levels	3	532.83	177.61	1.98 ns
Error	20	1,785.67	89.28	
Total	23	2,318.50		

TABLE IX

ANOVA Summary Table: S^--T Responses. Differences in d-Amphetamine Dosages with 5.0 mg/kg Amantadine Pretreatment

Source	df	SS	MS	F
Amphetamine dose levels	3	756.12	252.04	0.70 ns
Error	20	7,149.50	357.68	
Total	23	7,905.62		

TABLE X

ANOVA Summary Table: S⁻-T Responses. Differences in d-Amphetamine Dosages with Saline Pretreatment

Source	df	SS	MS	F
Amphetamine dose levels	3	3,382.73	1,127.57	6.80*
Error	20	3,318.90	165.94	
Total	23	6,701.63		

*p<.01

TABLE XI

ANOVA Summary Table: S⁻-I Responses. Differences in d-Amphetamine Dosages with Saline Pretreatment

Source	df	SS	MS	F
Amphetamine dose levels	3	1,260.46	420.15	5.71*
Error	20	1,472.50	73.62	
Total	23	2,732.96		

*p<.01

TABLE XII

ANOVA Summary Table: $\bar{S}$-T Responses. Differences in d-Amphetamine Dosages with 2.5 mg/kg Amantadine Pretreatment

Source	df	SS	MS	F
Amphetamine dose levels	3	943.17	314.39	5.39*
Error	20	1,167.33	58.37	
Total	23	2,110.30		

*p<.01

TABLE XIII

ANOVA Summary Table: $\bar{S}$-I Responses. Differences in d-Amphetamine Dosages with 2.5 mg/kg Amantadine Pretreatment

Source	df	SS	MS	F
Amphetamine dose levels	3	586.46	195.49	5.32*
Error	20	734.50	36.72	
Total	23	1,320.96		

*p<.01

TABLE XIV

ANOVA Summary Table: S^{-}-T Responses. Differences in d-Amphetamine Dosages with 7.5 mg/kg Amantadine Pretreatment

Source	df	SS	MS	F
Amphetamine dose levels	3	2,130.46	710.15	7.10*
Error	20	1,999.50	99.98	
Total	23	4,129.96		

*p<.01

TABLE XV

ANOVA Summary Table: S^{-}-I Responses. Differences in d-Amphetamine Dosages with 7.5 mg/kg Amantadine Pretreatment

Source	df	SS	MS	F
Amphetamine dose levels	3	1,064.50	354.83	10.28*
Error	20	690.00	34.50	
Total	23	1,754.50		

*p<.01

with 2.5 mg/kg amantadine, the results of the control treatment (saline) differed significantly ($p<.05$) from the results of treatment with either 0.5 or 1.0 mg/kg d-amphetamine. There were no significant differences between treatments of either 0.75 d-amphetamine or saline, or between 0.75 mg/kg d-amphetamine and the other d-amphetamine dosages.

The fact that there is no significant interaction between the d-amphetamine groups and the amantadine treatments indicated that the amantadine treatments did not block the effects that are usually noted following administration of d-amphetamine in animals trained on SDR. In fact, aside from the 5.0 mg/kg amantadine pretreatment group, the results of the study merely further support the previous finding that d-amphetamine enhances learning of the discrimination in the procedure used here. Perhaps the most interesting finding is that of non-significant differences between the d-amphetamine treatments following pretreatment with 5.0 mg/kg amantadine. This might lead one to conclude that this pretreatment did, in fact, block the effects of the d-amphetamine treatments. A closer examination of the data (Table XVI) indicates there is some form of interaction taking place following this pretreatment, but whether it is a blockage of d-amphetamine's effect or merely a disruption of the operant behavior is debatable. The results following pretreatment with 5.0 mg/kg amantadine revealed an increase in the number of errors after the 0.5 and 1.0 mg/kg d-amphetamine treatments. Had this been the only observable difference it might be more readily concluded that pretreatment with 5.0 mg/kg amantadine blocked the central effects of

TABLE XVI

Table of Means for All SDR Measures
for All Drug Combinations

d-Amphetamine Dose Levels	Amantadine Dosages			
	Saline	2.5	5.0	7.5
	- - - - - - - mg/kg - - - - - - -			
S+R responding				
0.50	99.0	94.8	92.5	96.7
0.75	99.2	97.5	92.0	94.0
1.00	98.8	82.3	75.8	99.5
Saline	98.1	97.3	98.5	97.5
S⁻T reponding				
0.50	14.8	8.3	20.7	11.5
0.75	10.7	12.7	12.7	5.3
1.00	7.3	8.3	19.7	8.7
Saline	37.7	23.7	28.5	29.7
S⁻I responding				
0.50	6.8	6.8	10.5	7.0
0.75	9.2	9.2	8.7	4.7
1.00	6.8	6.5	12.8	4.7
Saline	24.1	18.8	21.0	20.7
ITI responding				
0.50	25.5	21.1	41.1	25.3
0.75	16.5	15.0	16.3	11.8
1.00	22.8	36.1	34.8	9.7
Saline	25.7	21.1	33.3	28.9

d-amphetamine. However, S^+ responding was also affected. A decrease in S^+ responding was noted for all d-amphetamine treatments following pretreatment with 5.0 mg/kg amantadine. This finding, together with the above reported effect on S^--I responding, leads to the conclusion that this interaction is more one of disruption of the operant response rather than blockage of the effects of d-amphetamine.

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of this series of studies was to determine the dose-effect relationships of d-amphetamine and amantadine hydrochloride and to determine what effects the interaction of these two compounds would have on acquisition. Experiment I indicated that 0.50 mg/kg d-amphetamine closely approximates the dosage which provides for optimal performance on SDR. Dosages of 1.0 mg/kg and 2.0 mg/kg proved to be disruptive for performance on SDR. Experiments II and III investigating the dose response relationship of amantadine hydrochloride, indicated that dosages of 10 mg/kg and above resulted in detrimental effects on SDR performance, while dosages below 10 mg/kg seemed to have negligible effects on the task. In a general sense, the results from the final experiment indicate that amantadine hydrochloride did not block the effects of d-amphetamine on learning. To be more specific, in some instances, the interaction of the two drugs seems to be synergistic in nature rather than inhibitory.

These results do not imply that previous findings which reported inhibition of the central effects of d-amphetamine were in error; nor do they imply that the method employed was faulty. It has previously been demonstrated that the repeated acquisition method, using serial reversal discriminations, is a sensitive and reliable method for determining drug effects on learning (Calhoun and Jones, 1974). In addition, the method has been shown to effectively

demonstrate the effects of drugs on learning in this series of studies.

The results of the control injections for amantadine across the various amphetamine dosages differ somewhat from the results of Experiment I. This effect is possibly due to the extra handling and the second injection which were necessary in Experiment IV. Both handling and receiving injections are, at times, traumatic for an animal and could result in a physiological change (biochemical) which might result in some interaction of the drug administered and the physical state of the animal. A second possible reason for this difference is that of group differences. Generally speaking, when a well-controlled behavioral baseline such as the one employed here is used for drug experimentation, additional subjects add little information. However, it is possible that this effect is due to a sampling problem, and that a larger number of subjects would provide a more conclusive dose-response curve for d-amphetamine.

A second point which would be interesting to investigate concerns the dose-effect curve for amantadine hydrochloride. Most drugs can be administered at different levels to produce different effects, and it is not uncommon to find that a complete range of dosages results in a dose-response curve with a multiphasic pattern. The dosages tested for amantadine revealed that higher dosages appear to disrupt performance. The second range of dose levels tested indicated little, if any, change. For the purpose of this series of studies, this was determined to be the lower limit of effect for amantadine hydrochloride. However, it is possible that lower dose levels would produce even a different effect. If these were tested

and found to be stimulant in nature it would be helpful in explaining some of the results in Experiment IV which indicate that the interaction of amphetamine and amantadine was synergistic and stimulatory.

The results of Experiment IV indicated that there was little interaction between 2.5 mg/kg amantadine hydrochloride and the various amphetamine dosages. It appears that, following 5.0 mg/kg amantadine pretreatment, there is a disruption of discrimination which is greatest with the 1.0 mg/kg d-amphetamine combination. The results following pretreatment with 7.5 mg/kg amantadine are interesting. It appears that following this pretreatment all combinations with d-amphetamine result in an enhancement of discrimination. This finding is not in agreement with others reported herein. However, it is important to recall that previous studies reporting inhibition of d-amphetamine's effect following amantadine pretreatment were concerned with motor activity. It is possible that this dose level of amantadine did block the hyperactivity noted after d-amphetamine administration thereby increasing attentiveness to the task.

A second possible explanation for the results following 7.5 mg/kg amantadine pretreatment concerns the hypothesis that stimulant drugs increase motivational levels (Handley, 1971). Therefore, the 7.5 mg/kg dose level of amantadine may be high enough to bring the increased motivational level of the subjects following amphetamine administration into a more optimal level for discrimination tasks.

A final possibility as an explanation for the results following pretreatment with 7.5 mg/kg amantadine concerns the reports by various authors (Herblin, 1972; Fletcher and Redfurn, 1970; Vernier et al.,

1969) that amantadine hydrochloride increases the effect of norepinephrine while at the same time inhibiting reuptake of norepinephrine into nerve endings. The most plausible explanation for this evolves from reports concerning the early use of amantadine hydrochloride. Amantadine was first used as a prophylactic treatment against Type A-2 influenza. Its use in preventing this viral infection was based on its ability to adsorb onto cells, thereby preventing viral absorption. Given the propensity of amantadine to coat cell membranes, it is plausible that amantadine blocks the reuptake of norepinephrine at the presynaptic site leading to a prolonged or increased norepinephrine effect on postsynaptic receptors. Administration of amantadine merely serves to increase the amount of NE released from presynaptic stores and permits even greater norepinephrine effect on receptor sites.

From this discussion, it should be clear that drug interactions are very complex in nature. However, the explanations suggested here for the results obtained in this series of studies do provide several possibilities as to interactions occurring. It is apparent that, due to the complex nature of a study such as this, further research should be pursued for the results of this study to be conclusive. The explanations offered here provide direction for future research concerning the interactions of d-amphetamine and amantadine hydrochloride.

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