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The Effect of Two Levels of Δ9 THC on State-Dependent Learning in Rats

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This experiment was performed to determine whether a state-dependent learning effect is produced when rats are under the influence of Δ^9 THC. A latent learning procedure utilizing a Lashley III maze was used. Latent learning paradigms offer one a variety of measures not available when using an operant procedure.

Forty-five female rats were run; five in each of nine conditions. Each set of nine rats was run as follows:

Days 1-5. Each rat received an injection of 0.0 mg/kg, 0.4 mg/kg, or 1.0 mg/kg of Δ^9 THC. One-half hour later the

rat received one-half hour of exposure in a Lashley III maze. The rats received the same doses for each of these five days.

Days 6-7. The rats were fed to maintain 80 percent ad lib weight.

Days 8-9. The rats received their test condition dosage of Δ^9 THC (0.0 mg/kg, 0.4 mg/kg, or 1.0 mg/kg) and were then fed.

Day 10. The rats were injected with the test condition dosage and were then placed in the maze. Food was offered as reinforcement. Time-per-trial and errors-per-trial were recorded. One trial was run.

Day 11. The rats were given the test condition dose and placed in the maze for four reinforced trials.

The expectation was that a state-dependent learning effect would be evidenced by low scores in the 0.0 mg/kg-0.0 mg/kg, 0.4 mg/kg-0.4 mg/kg, and the 1.0 mg/kg-1.0 mg/kg conditions. This would result in a significant interaction effect when a three-way analysis of variance was performed on the data. This statistical effect did not happen.

An attempt was made to determine why the results were insignificant. The results did not replicate an earlier study done by Burke (personal communication). Burke obtained significant differences between the control group and the drug group. Doses ranged from 0.4 mg/kg to 2.0 mg/kg of

Δ^9 THC in the Burke experiment. Differences between the present study and the Burke study were explored. They were:

(1) Difference in sex of the rats.

(2) Ethanol in the solution used in the present study.

(3) This study used Δ^9 THC and the Burke study used Marijuana Extract Distillate (MED).

It was concluded that the sex difference and the presence of ethanol were not factors that differentiated the present study from the Burke study. It was not clear whether MED is effective at lower doses than Δ^9 THC. Literature on the synergistic effect of the components of marijuana other than Δ^9 THC was conflicting. What was clear was that the minimum dose of Δ^9 THC needed to produce a discriminable effect on behavior is 1.3 mg/kg. The maximum dose in the present procedure was 1.0 mg/kg. The suggestion was made that the present study be re-run with higher dose levels.

THE EFFECT OF TWO LEVELS OF Δ^9 THC ON STATE-DEPENDENT LEARNING IN RATS

by

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CHAPTER I

INTRODUCTION

There is much interest in the effects of Cannabis sativa on both the physiology and psychology of animals, man included. Experiments on the effect of marijuana on learning in animals is one area that has yielded confusing results.

Most experiments studying the effects of marijuana on behavior use some derivative of Cannabis sativa. Some examples of these derivatives are marijuana extract distillate (MED), alcoholic marijuana extract, and delta 9 tetrahydrocannabinol (Δ^9 THC) which was previously labelled delta 1 THC. The first two substances contain the complete marijuana substance. The Δ^9 THC is one molecule of all the possible molecules in crude marijuana. Synthetic marijuana (DMHP) is a less commonly used experimental substance. The common link between these four solutions is that when used for experimental purposes they are assayed for Δ^9 THC content. This is done because it is assumed that Δ^9 THC is the precursor of the pharmacological effect of Cannabis sativa.

A number of learning studies with marijuana have been done within an operant paradigm. The operant set-up seems

to be the most widely used method because of its refined techniques and technology. In an operant study an animal has to emit a certain behavior such as bar pressing. When the behavior occurs the animal receives reinforcement such as food. The measure of learning is the rate of the behavioral response. In experiments done with an operant procedure there seems to be a decrease in response rate caused by administration of marijuana. Frankenheim (1971) worked with pigeons and gave doses of Δ^9 THC ranging from 0.3 mg/kg to 3.0 mg/kg. It was found that the key pecking response measured on a multiple schedule declined dependent on the size of the dose. The larger the dose, the lower the response rate. Scheckel (1968) worked with monkeys in an avoidance procedure. The results showed reduced rates of response with moderate doses of Δ^9 THC (4 mg/kg-8 mg/kg) and increased rates of response with high doses (16 mg/kg-64 mg/kg). A third study by Boyd (1963) was done with rats and DMHP. A decrease in response rates on almost all schedules was found when the drug was administered.

But one problem that is present when an operant procedure is used to test for learning is the possible effect of Δ^9 THC on the activity levels of animals. If Δ^9 THC lowers the rate of all activity, then lower response rates on operant tasks might be an invalid measure of the drug's effect on learning. Barry and Kubena (1970) found that low levels of Δ^9 THC (4 mg/kg) produced initial excitation, but

the eventual consequence was a decrease in spontaneous activity in rats. Higher doses of 16 mg/kg produced an immediate decrease in activity.

The use of a latent learning paradigm might be one way to eliminate the problems in measurement caused by Δ^9 THC reducing the activity levels of animals. In a latent learning procedure the animal is allowed to explore the maze and then it is tested for learning by seeing how well it runs the maze in a reinforcement situation. There are several dependent variable options in a latent learning set-up such as errors and time per trial. These measures, especially errors, do not put the emphasis on rate of response but on accuracy as a measure of learning. There have been several experiments done with marijuana in a latent learning framework. Orsingher and Fulginiti (1970) found that rats given 10 mg/kg of Δ^9 THC made more errors in a Lashley III maze than rats who were given a placebo. Burke (personal communication) also ran rats in a Lashley III maze. Three low level doses of MED were administered before allowing the rats to explore the maze. The doses were assayed at 0.4 mg/kg, 0.8 mg/kg, and 2.0 mg/kg of Δ^9 THC. A 0.0 mg/kg dose was also given as a control dose. When subsequently tested in an undrugged state, the rats that had been drugged during the maze exposure period made significantly more errors than the rats that had received the control substance. There was no difference in errors among the three drug levels. Food

was used as reinforcement during the test trials. Wetle (1971) found that marijuana does not start to affect eating behavior till doses of 1.5 mg/kg of Δ^9 THC. At that point appetitive behavior decreases. This did not seem to be a factor in the Burke study.

The past two studies point towards the same conclusions one would draw from the operant studies; marijuana seems to hinder the efficiency of learning. But neither of the experiments cited have taken into account the possibility that state dependent learning might be occurring. Overton defined state dependent learning as follows:

> State dependent learning (StD) is said to be present when an alteration of a learned response is due to a change of state per se. Such state change can be obtained in three ways: from a drugged (D) to a non-drugged (ND) state; from a non-drugged to a drugged state; and from one drug state to another (1966, p. 87).

In the Orsingher and Fulginiti (1970) study, all of the rats in the maze exposure condition, during which they explored the maze, were undrugged. In the Burke (1972) study, all of the rats in the test condition, during which errors were recorded and food was offered as reinforcement, were undrugged. What is needed is an experiment where there is control for the possibility of state dependent learning. A certain number of test condition trials should be presented during which the animals are in the same drug states as during the maze exposure condition.

Overton (1971) provides an overview of the discriminative control that drug states exert on behavior. Most of

the experiments have been done in a T maze. It has been shown that reversal learning can be aided by switching drug states. Overton cites many drugs that exert this discriminative effect on behavior; but Δ^9 THC was not one of them. On the other hand, Jarbe and Henriksson (1973), using Δ^9 THC (5 mg/kg) found that reversal learning in a T maze was state dependent in the rat.

The present procedure was a partial replication of the Burke (personal communication) study; but provided a range of drug doses in the maze exposure and maze test conditions. The doses were 0.0 mg/kg, 0.4 mg/kg, and 1.0 mg/kg of Δ^9 THC in its isolated form. This type of a three by three experimental design would provide information on any state dependent effect. The low doses were chosen because of the results of the Burke (1972) study where a difference was found between the control dose and the 0.4 mg/kg dose of Δ^9 THC.

The hypothesis of this experiment was that a state dependent effect would be evident; as measured by errors per trial or time per trial. There would be a diagnonal of low values on the matrix in the 0.0 mg/kg-0.0 mg/kg cell, the 0.4 mg/kg-0.4 mg/kg cell, and the 1.0 mg/kg-1.0 mg/kg cell (Table I). Statistically, it was expected that there would be a significant interaction effect when a three-way analysis of variance was performed on the data.

TABLE I

THE EXPERIMENTAL DESIGN OF THIS STUDY

			Maze Test		
			Δ^9 THC 0.0 mg/kg	Δ^9THC 0.4 mg/kg	Δ^9 THC 1.0 mg/kg
Maze Exposure	Δ^9 THC	0.0 mg/kg	5	5	5
	Δ^9 THC	0.4 mg/kg	5	5	5
	Δ^9 THC	1.0 mg/kg	5	5	5

NOTE: The numbers in each cell represent the n.

CHAPTER II

METHOD

The subjects were 45 adult female Sprague-Dawley rats and they were run in a Lashley III maze. The rats were divided among nine conditions; five rats per condition. Table I illustrates the three by three experimental design. The rats were kept in an isolated room and the day-night cycle was controlled such that the lights went off one hour before the start of injections. The rats were also run in darkness. The doses of Δ^9 THC were administered by means of intraperitoneal injections. The Δ^9 THC was dissolved in a solution of ethanol, saline and TWEEN 80. The control solution was identical to the drug solution except for the omission of the Δ^9 THC. The rats were injected by one experimenter one-half hour prior to being placed in the maze. The drug's full effect lasts approximately one hour. A second experimenter put the rats in the maze and recorded the data. This double blind procedure eliminated the problem of experimenter bias because the person recording the data had no dosage information. The rats were deprived of food to 80 percent of their free feeding weight previous to the start of the experiment. Each animal was kept at this weight throughout the study by daily weighings and

individual feedings. The animals were fed with wet mash during the entire study. The procedure took 11 days to run for each set of nine rats. Each set was run as follows:

Days 1-5. During this exposure period the rats received an injection of 0.0 mg/kg, 0.4 mg/kg, or 1.0 mg/kg of Δ^9 THC each day. The dose for each individual rat was the same for each of these five days. The rats were then allowed to explore the maze for one-half hour on each day with no food in the goal box. After running they were fed in their home cages.

Days 6 and 7. This was a two-day break during which the rats received no injections. They were weighed and fed to maintain them at 80 percent of their ad lib feeding weight.

Days 8 and 9. These days were a time to acclimate the rats to their test condition dosage. Each day the rats were weighed, received their test condition dosage, and were fed. The rats were not put into the maze on these days.

Day 10. This was the first test condition day. The rats received their test dosage of Δ^9 THC. Then each rat was placed in the maze for a test run. Wet mash was placed in the goal box for reinforcement. The amount of time it took each rat to run the trial as well as the number of errors made were recorded. An error was defined as a rat placing one of its front paws in an incorrect alley or turning around and running back towards the start box. A rat

was considered turned around when one of its front paws touched the ground behind it. The rats were not fed on this day except for the wet mash consumed during the test run.

Day 11. The final four test runs were done on this day. The animals received their test dose and had four consecutive trials in the maze. Data were once more collected on time per trial and errors. There was approximately 1 gram of wet mash in the goal box. A trial was considered finished when the wet mash was consumed.

A pilot study consisting of six sets of rats (nine rats per set) was run before this final procedure was solidified. During the first few pilot series all of the test trials were run on one day (day 10). In addition the rats were maintained at 80 percent of their ad lib feeding weight by giving them dry food pellets. The wet mash was introduced two days before the test trials were run. The mash was used as food reinforcement in the maze because it is more aromatic than dry food.

During these initial pilot runs there was a problem with the rats failing to run to criterion during the test condition trials. Criterion is defined as a complete run through the maze to the goal box and consumption of the wet mash. The rats would run the first one or two trials of the required five runs and then start to avoid the goal box.

It was decided that this behavior was a case of neophobia. Neophobia is the avoidance of a novel stimulus.

The wet mash in the maze environment was a novel stimulus to the rat and thus was avoided.

Two procedural changes were introduced to solve this problem. The first change was to start feeding the rats exclusively on wet mash. The mash was introduced on the same day food deprivation began. This was usually one week previous to the first maze exposure day (day 1).

The second change was to use two test condition days (days 10 and 11) instead of only one. On day 10 one test trial was run and the animal was put on complete fast until the next day. On day 11 the final four trials were completed. It was felt that this change would enable the rats to acclimate to the new maze environment which included the wet mash. Also the animal would enter day 11 after total food deprivation for one day. Burke (personal communication) used the same two-day test condition when that study encountered similar problems.

These changes greatly reduced the number of uncompleted test trials. There were still occasional trials when the rats would spend 45 minutes or more without reaching the goal box. It was decided that there would be a 15-minute-per-trial limit to avoid this problem.

CHAPTER III

RESULTS

The data for the experiment are summarized in Tables II and III. The first number in each cell of the matrices is the grand means of the 25 trials per condition. The second number in each cell is the standard deviation for that condition.

TABLE II

THE RESULTS FOR THE DEPENDENT VARIABLE OF TIME PER TRIAL

			Maze Test		
			Δ^9 THC 0.0 mg/kg	Δ^9 THC 0.4 mg/kg	Δ^9 THC 1.0 mg/kg
Maze Exposure	Δ^9 THC	0.0 mg/kg	4.94±4.58	6.25±3.4	5.34±3.73
	Δ^9 THC	0.4 mg/kg	2.88±.82	4.66±3.98	1.96±1.12
	Δ^9 THC	1.0 mg/kg	5.81±5.58	6.24±4.24	4.95±4.5

NOTE: The first number in each cell is the grand mean for that condition and the second number is the standard deviation for that cell. All data are listed as running time in minutes with associated standard deviation.

TABLE III

THE RESULTS FOR THE DEPENDENT VARIABLE OF ERRORS PER TRIAL

			Maze Test		
			Δ^9 THC 0.0 mg/kg	Δ^9 THC 0.4 mg/kg	Δ^9 THC 1.0 mg/kg
Maze Exposure	Δ^9 THC	0.0 mg/kg	6.76±3.14	7.68±1.67	7.24±3.4
	Δ^9 THC	0.4 mg/kg	6.80±2.16	7.36±3.58	6.20±1.3
	Δ^9 THC	1.0 mg/kg	6.36±2.06	9.4±2.53	9.08±3.25

NOTE: The first number in each cell is the grand mean for that condition and the second number is the standard deviation for that cell.

Table II is the matrix for the dependent variable of time per test trial. The three fastest grand mean times were in the second row of the matrix. These conditions were the ones where the rats received 0.4 mg/kg of Δ^9 THC during the maze exposure condition. The times were 2.88 minutes per trial in the 0.4 mg/kg-0.0 mg/kg condition, 4.66 minutes per trial in the 0.4 mg/kg-0.4 mg/kg condition, and 1.96 minutes per trial in the 0.4 mg/kg-1.0 mg/kg condition.

A three-way analysis of variance (ANOVA) was done on the raw data used to obtain the means in Table II. The

results of this analysis are summarized in Table IV. There were no statistically significant differences among the conditions or from the interaction of conditions. The most important point of this analysis is in the row effect. As noted in the last paragraph, there was a row of lower times that distinguished the 0.4 mg/kg maze exposure level (row 2) from the other two exposure dose levels. This difference did not hold up statistically. The F for row effect was 1.90. An F of 3.15 would have been needed to obtain a significant difference at the .05 level. A reexamination of Table II points out that the standard deviation of times within each cell are very large. The range of standard deviations was from a low of 0.82 to a high of 5.58. This large variance of scores within each cell eliminated the possibility of obtaining statistically significant results.

The only statistically significant result in the ANOVA on time was found within subjects. This information is also contained in Table IV. There was a significant decrease in time per trial to the .01 level (F=3.99). These results are consistent with one's expectations of a learning study. In other words; as more trials were run the time for each succeeding trial decreased.

Table II is the matrix for the dependent variable of errors per test trial. There seems to be no hint of a pattern in this matrix. The only values that stand out are the 1.0 mg/kg-0.4 mg/kg cell and the 1.0 mg/kg-1.0 mg/kg cell,

TABLE IV

THREE-WAY ANALYSIS OF VARIANCE ON TIME PER TRIAL MEASUREMENT

Source	DF	F	
Between subjects	44		
Rows	2	.69	
Columns	2	1.90	Non-significant
Rows X columns	4	.10	
Error between	36		
Within subjects	180		
Time	4	3.99	Significant
Columns X time	8	.63	
Rows X time	8	1.74	Non-significant
Columns X rows X time	16	.73	
Error within	144		

where the values were 9.4 and 9.08 errors per test trial respectively. This might indicate the beginning of an effect at higher dose levels; but one that certainly lacks significance at this level. This lack of statistical significance is obvious in Table V, which is the three-way ANOVA for the errors per trial measurement. There was an F of 0.75 for the row effect and 0.81 for the interaction effect. As expected from the initial inspection of the data, none of these F ratios approach the 3.15 necessary for significance at the 0.5 level.

Tables VI and VII are the results of the one factor ANOVAs done for time and errors. The comparison is between the rats in the three exposure conditions in relation to

TABLE V

THREE-WAY ANALYSIS OF VARIANCE ON ERRORS PER TRIAL MEASUREMENT

Source	DF	F
Between subjects	44	
Columns	2	.98
Rows	2	.75
Columns X rows	4	.81
Error between	36	
Within subjects	180	
Time	4	1.52
Columns X time	8	1.05
Rows X time	8	1.01
Columns X rows X time	16	1.19
Error within	144	

TABLE VI

ONE-FACTOR ANALYSIS OF VARIANCE ON THE TIME PER TRIAL MEASUREMENT

Source	DF	F
Treatments	2	.57
Errors	12	

NOTE: Column one (0.0 mg/kg test dose).

TABLE VII

ONE-FACTOR ANALYSIS OF VARIANCE FOR ERRORS PER TRIAL MEASUREMENT

Source	DF	F
Treatments	2	.05
Error	12	

NOTE: Column one (0.0 mg/kg test dose).

their performance at the 0.0 mg/kg dose level during the test condition trials. Essentially only the first column of the matrices represented by Tables II and III were analyzed. The rationale for these analyses was to provide a comparison between the present study and the one done by Burke (personal communication). In that study four different doses of MED (assayed for Δ^9 THC content) were administered during the maze exposure condition. The doses were 0.0 mg/kg, 0.4 mg/kg, 0.8 mg/kg, and 2.0 mg/kg. No drug was administered during the test condition. In the Burke study it was found that there was a significant decrease in performance, as measured by errors, between the non-drug group and the drug group. There was not a significant difference between the three drug levels. In the present procedure there were no significant differences on time per trial or errors per trial between any of the three different maze exposure doses of Δ^9 THC. The results of the present experiment conflict with the results of the Burke (1972) experiment.

CHAPTER IV

DISCUSSION

The primary expectation at the outset of this study was that a state dependent learning effect would be evident in the results. An ideal matrix would have had the lowest scores (time or errors) for each successive row in the 0.0 mg/kg-0.0 mg/kg cell, the 0.4 mg/kg-0.4 mg/kg cell and the 1.0 mg/kg-1.0 mg/kg cell. This would appear as a diagonal of low values on both the time and error matrices. A glance at Tables II and III indicates that there was no such effect. The three-way ANOVAs further support this conclusion. The expectation was that there would be a significant interaction effect if the state dependent hypothesis held. This did not happen.

A secondary expectation involved column one of the matrix. These are the three conditions where no dose was administered during the test condition. The column was a partial duplication of Burke's (1972) experiment. As noted earlier there was a significant decrease in performance between the non-drug and the drug group in the Burke experiment, with no difference among the different levels of drugged animals. In the present study there was no loss of performance in either the time or the errors per trial.

This is evident by either a quick inspection of Tables II and III or by consulting Tables VI and VII which are the one-way ANOVAs for these data.

The task at this juncture is to determine why the results of this study proved so inconclusive. Although this procedure was a partial replication of the Burke (personal communication) study, there are some differences between the two that may point towards an answer to this question.

(1) The present study used female rats; the Burke study used male rats.

(2) The present study used a solution of Δ^9 THC mixed with ethanol and TWEEN 80. The Burke study used MED dissolved in TWEEN 80 which was assayed for Δ^9 THC.

These two differences point out four potential areas that may have affected the measurement of dependent variables. These four areas will now be examined.

The first difference between the two experiments is the question of differing male and female responses to marijuana. Cohen, Barnes, et al. (1972) administered oral doses of MED ranging from 5 mg/kg to 40 mg/kg to male and female rats. There were six behavioral tests performed on the rats. An example was the drop test. Each rat was dropped from a specified height and the independent variable was how much time passed before the rat started to move. The conclusion was that the behavior ratings for the female rats were significantly higher than the ratings for the male

rats. This indicates a stronger reaction in female rats when equivalent doses were given. For example, it was concluded that the behavior of males receiving 20 mg/kg doses was comparable to the behavior of females receiving 5 mg/kg. All of the doses were assayed for Δ^9 THC.

Several follow-up studies were done to determine if there was a physiological basis for the behavioral difference between the sexes. Cohen, Williams, et al. (1974) administered 2 mg/kg of Δ^9 THC to male and female rats. They examined the rats 45 minues after administering the drug. A significantly higher level of Δ^9 THC, measured radioactively, was found in the brain, liver, muscle, and plasma of the female rats. A further study by Cohen, Barrat, et al. (1973) found that injection of testosterone in female rats and castration of male rats would reverse this effect.

There seem to be well-documented differences between male and female rat reactivity to Δ^9 THC. Females are more sensitive to equivalent doses. In terms of the present study, this effect would indicate that the female rats should have exhibited an effect more readily; had an effect been present. When this study is compared to the Burke study, it would be expected that the effect noted by Burke would be exaggerated by the use of female rats in this experiment. In fact the opposite was true. This means that another variable, other than the male-female difference, was active on the results of one or both of the two experiments.

The second avenue to be explored is the comparison of the drug solution used in this study and the solution used in the Burke (1972) study. The presence of ethanol in the solution used in this study brings up the possibility of a dual effect. The Δ^9 THC solution was mixed twice during the course of this experiment. The Δ^9 THC was received from the Federal government dissolved in ethanol. Care was taken to evaporate as much of the ethanol as possible before mixing a working solution. The ethanol dose was 42 mg/kg in the first solution, which is the one which had a slightly higher ethanol level.

Three studies that examined the effect of ethanol on behavior were consulted. Arvola et al. (1958) administered ethanol through a stomach tube. The doses ranged from 900 mg/kg to 8100 mg/kg. The rats were presented with many activities such as a righting reflex in water and sliding off of a tilted board. In only one activity did the low dose group exhibit a significant difference from the performance of the control group. This low dose of ethanol was 20 times the size of the high dose in the present experiment.

Two further studies, Belknap et al. (1975) and Skurdal et al. (1975) injected the ethanol intraperontineally. Both procedures injected low doses of 1200 mg/kg. In the former study the ethanol impaired the behavior of the rats as measured by the frequency at which their feet slipped through

the floor of the cage. In the latter study the low dose significantly affected the acquisition of escape behavior.

Barry (1973) did an overview of work that had been done to classify drugs according to their discriminable effects in rats. The studies used the drugs as a discriminative stimulus (S^D) for reinforcement. The levels at which these drugs yielded significant behavioral differences was noted. The ED_{50}, which is the effective dose with a 50 percent probability of eliciting the desired response, was listed for several experiments involving ethanol. Barry and Kubena (1969) found an ED_{50} of 525 mg/kg and Krimmer and Barry (1966) found an ED_{50} of 617 mg/kg. These doses are in excess of ten times the size of the largest dose of ethanol in the present experiment.

It certainly seems that the amount of ethanol used in the solutions in this experiment was well below the level that would have had any behavioral effect on the rats. There remains the possibility of a synergistic effect between marijuana and ethanol. There exists no evidence of this in the present study as displayed by the non-significant differences among all the drug and non-drug groups.

Another difference between the solution used in this study and the one used in the Burke study is that this study used a Δ^9 THC mixture and the latter study used MED. This then brings up the question of whether there are any other active components in marijuana other than Δ^9 THC that might

affect behavior. As previously mentioned when discussing the effect of ethanol on behavior, Barry and Herbert (1972) classified various drugs according to their discriminable effects on behavior in rats. They also examined four marijuana solutions: two of Δ^9 THC, one of MED, and one of alcoholic marijuana extract. The two Δ^9 THC mixtures and the MED had an ED_{50} range of 1.3 mg/kg to 1.7 mg/kg. But the alcoholic marijuana extract had an ED_{50} of 0.58 mg/kg. The authors then quote an associate who had been using Dimethylheptyl (DMH) which is an analog of $\Delta^{6a_{10a}}$ THC. This colleague stated that the DMH was more than 200 times as potent as the Δ^9 THC solution.

There are certainly conflicting data here because one extract, MED, seems to be equivalent in potency to Δ^9 THC preparations; yet the other extract, the alcoholic marijuana extract, seems much more potent than the Δ^9 THC. A further study by Kubena and Barry (1972) replicates these findings. The ED_{50} of the two Δ^9 THC preparations and the MED ranges from 1.4 mg/kg to 1.94 mg/kg. The ED_{50} for the alcoholic marijuana extract was 0.51 mg/kg.

This confusion is further compounded by two further studies. Mechoulam and Shani (1970) used an etherized hashish extract with monkeys. The only compound to cause a change in activity in the animals was the Δ^9 THC fractionated from the hashish but did not affect the behavior of the monkeys.

Another study by Gill et al. (1970) found results that are opposite to the previous study. The conclusion of this procedure points to at least five pharmacologically active components of marijuana; three were water soluble and two were fat soluble. It was found that these compounds affected specific physiological reactions such as heart rate, salivary secretion, and acetylcholine output from the parasympathetic nervous system. It is expected that physiological reactions of this nature would also have a noticeable effect on behavior.

What can be concluded from these conflicting studies? The present study gauged the dose levels on the experience of Burke (1972). In that experiment MED was used and had a significant effect on behavior. There is a possibility that the administration of a marijuana extract has a stronger effect than the administration of the isolated Δ^9 THC compound. If this is true, it would help to explain the lack of significant results in the present procedure. Unfortunately, because of the opposing views about the active ingredients of marijuana it would be unwise at this time to attribute the present results to this factor.

What is most likely the outstanding reason for the non-significance of the present procedure is contained in the article by Barry and Herbert (1972). As previously noted, a comparison was made of the two Δ^9 THC compounds and two marijuana extracts. There were conflicting data about

the potency of the two extracts as compared to the Δ^9 THC compounds. But there was consistency between the two Δ^9 THC preparations. One compound had an ED_{50} of 1.4 mg/kg to 1.9 mg/kg and the other had an ED_{50} of 1.3 mg/kg to 1.6 mg/kg. What these results suggest is that the high dose (1.0 mg/kg) used in the present study was at least 30 percent too low to achieve a behavioral effect. Not only is this implication supported by this overview article, but it is further indicated by an examination of several other procedures in which Δ^9 THC was used.

Barry and Kubena (1972) did a more thorough exploration of the behavioral effects of marijuana. Once again Δ^9 THC was used as a S^D for either avoidance or approach behavior. The behavior was established at 4.0 mg/kg and then the doses were reduced to 2.0 mg/kg, 1.0 mg/kg and finally 0.5 mg/kg. In this more extensive procedure the ED_{50} of Δ^9 THC was established at 1.4 mg/kg. The effect of Δ^9 THC seemed to dissipate somewhere between 2.0 mg/kg and 1.0 mg/kg. At 2.0 mg/kg the appropriate drug response (approach or avoidance) occurred 80 percent of the time. At 1.0 mg/kg the drug response occurred only 36 percent of the time.

Henriksson and Jarbe (1972) used Δ^9 THC as a S^D for rats learning a T maze. They found significant results using doses of 5.0 mg/kg and 10 mg/kg.

Another experiment by Barry and Kubena (1971a) explored the relationship between the pharmacological and

behavioral effects of Δ^9 THC. Daily doses of Δ^9 THC ranging from 2 mg/kg to 16 mg/kg were administered to rats for one week. Strong pituitary and adrenal activation was noted as measured by assays of plasma corticosterone levels. This pharmacological effect was detected at all dose levels. A behavioral effect was also present at all levels except the low doses of 2.0 mg/kg and 4.0 mg/kg.

Other experiments that were referred to that tested for dissociation of learning or maze behavior under the influence of marijuana, such as Bueno and Carlini (1972) and Carlini and Kramer (1965), measured doses to be equivalent to 5 mg/kg or 10 mg/kg of Δ^9 THC.

In conclusion it can be stated that when the results of this study yielded insignificant results the question of why became important. Four areas were considered as possibly having an effect on the outcome of the experiment. Three of them were dismissed:

(1) Female rats were used in this study. All references pointed to a stronger pharmacological and psychological drug effect in females. This indicates that if an effect were in fact present it should have been more pronounced in female rats.

(2) Ethanol was present in the Δ^9 THC solution administered to the rats. It was concluded after examining the references that the level of alcohol was far below that which would have any effect on the behavior of the rats.

(3) A Δ^9 THC compound was used in this study rather than a complete marijuana extract. The references on the active components of marijuana were at best confusing. Some studies support the view that there are other active elements in marijuana other than Δ^9 THC or at least there is a synergistic effect. Other experiments insist that Δ^9 THC is the only active constituent of marijuana. Because of this widespread confusion; the fact that a complete marijuana extract was not used was discounted as a factor affecting the outcome of this study.

The fourth avenue of exploration proved to be the most fruitful. The literature consistently cited doses of Δ^9 THC from 1.3 mg/kg to 1.9 mg/kg as the ED_{50}. These previous findings point out the primary fault of the present experiment which was the lack of a potent dose of Δ^9 THC.

With this knowledge in hand a more realistic experimental design can be formulated. A four by four matrix can be envisioned with doses of Δ^9 THC ranging from 0.0 mg/kg to 5.0 mg/kg. In addition, this design could be repeated using MED. This type of double study would help to answer several questions raised in this discussion, such as realistic dose levels and the presence of other active ingredients in marijuana.

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