The effects of Δ^9 -tetrahydrocannabinol upon fear-motivated behavior in squirrel monkeys

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The present study investigated the effects of three doses (4.0, 8.0, 12.0 mg/kg) of Δ^9 -tetrahydrocannabinol (THC) upon the behavior of six squirrel monkeys subjected to a Sidman nondiscriminated avoidance schedule that superimposed conditioned stimuli (CS)—unavoidable shock pairings upon the ongoing avoidance behavior. All the animals demonstrated facilitated avoidance response rates and general motor activity during the aversive CS. Oral administration of THC produced avoidance decrements with a consequent increase in the number of shocks received only under the highest dosage (i.e., 12.0 mg/kg). All doses of THC reduced the facilitation of avoidance response rates normally noted during the aversive CS and severely reduced general motor activity. These results were interpreted to suggest that THC may reduce the level of fear normally generated during the presentation of an aversive CS previously associated with unavoidable shock.

Previous reports have indicated that marihuana compounds are not able to affect behavior under aversive control in a consistent manner. For example, Boyd, Hutchinson, Gardner, and Meritt (1963) have noted that dimethylheptyltetrahydrocannabinol enhances the conditioned suppression of barpressing behavior in rats. Similar results were obtained with Δ^9 -tetrahydrocannabinol (THC), the active constituent of marihuana, which produced longer drinking latencies (i.e., greater conditioned suppression) in rats shocked and retested under the drug (Robichaud, Hefner, Anderson, & Goldberg, 1973). Contrasting results, however, were reported by Gonzalez, Karniol, and Carlini (1972), who noted that rats treated acutely with marihuana extract demonstrated a reduction in the conditioned suppression of drinking behavior noted in the presence of a conditioned stimulus (CS) previously associated with electric shock. Furthermore, these authors noted that the rats treated with marihuana extract demonstrated decreased defecation during the CS when compared to control animals. These results were interpreted to suggest that marihuana compounds may retard the acquisition and retention of the conditioned emotional response (CER) by reducing the level of fear, thus facilitating the emission of ongoing behavior. Similar results were obtained by Abel (1969) who demonstrated that pyrahexyl, a marihuana homologue, partially blocked the suppressive effects of a CS previously paired with electric shock in a CER paradigm.

Since the existing literature, cited above, has produced conflicting results, it would appear that more research concerning the effects of marihuana compounds

upon fear-motivated behavior is in order. The present paper is an attempt to explore the effects of Δ^9 -THC upon behavior controlled by a modified, nondiscriminated Sidman avoidance schedule that contains CS-unavoidable shock presentations. Many investigators (Herrnstein & Sidman, 1958; Houser & Houser, 1973; Rescorla, 1967; Sidman, 1958; Sidman, Herrnstein, & Conrad, 1957) have reported that when an aversive CS previously associated with unavoidable shock is superimposed upon operant behavior maintained by a nondiscriminated avoidance schedule, response rate is elevated during the CS. This facilitation of rate during the CS is maintained even when baseline avoidable shocks are omitted during the CS (Houser, Reference Note 1), or when the avoidance response has been extinguished (Waller & Waller, 1973). This evidence has been interpreted as support for the two process-learning theories which postulate that Pavlovian conditioned responses motivate instrumental responses (Maier, Seligman, & Solomon, 1967). Thus, since both the instrumental avoidance response and the Pavlovian conditioned response are motivated by fear, combining the two responses in one behavioral schedule increases the level of fear during the Pavlovian CS, as indicated by the increased instrumental response rates. These elevated response rates during the CS, in turn, can be used as an index of fear motivation under drug or control conditions. The present report evaluated the effects of several doses of Δ^9 -THC upon the avoidance response rates of squirrel monkeys during both CS and non-CS segments of this modified schedule.

METHOD

Subjects

The subjects in this experiment consisted of six male squirrel monkeys (*Saimiri Sciureus*) ranging in weight from 795 g to

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1,022 g. All animals were housed in a colony where the lights were on for 12 h of each day (i.e., from 6:00 a.m. to 6:00 p.m.). Experimental testing took place between 8:00 a.m. and 2:30 p.m. Each animal was housed in a separate home cage in the colony and was placed in the restraint chair in the experimental testing chamber for only 2 h each day. Animals were then returned to their home cages where they had free access to food (Purina Monkey Chow) and water.

Apparatus

All animals were tested in one of two Plexiglas restraint chairs (Lehigh Valley Electronics, Model 142-10). Shock was applied to a shaved portion of the animal's tail via two brass electrodes that were placed on the skin surface. A plastic lock around the base of the tail and a rubber band around the two electrodes insured that the animal could not move the position of the electrodes during the session. The shock stimulus was provided by a high-voltage (1,780 V) ac source which placed a large resistance (540 K) in series with the animal's tail. Thus, minor fluctuations in tissue resistance would not affect current intensity to any appreciable degree. To further insure that the shock stimulus was constant between experimental sessions, resistance was monitored across the animal's tail by a Triplett (Model 630-A) multimeter before each session to insure that it remained at 3,200 ohms (± 100 ohms). This relatively constant resistance between sessions was made possible by applying small amounts of Redux electrode paste between each electrode and the animal's skin. A response lever (Lehigh Valley Electronics, Model 121-05) was located directly in front of the animal and required a downward pressure of 10 g to activate the microswitch. The houselight (TS-304-28 V dc) located directly above the animal's head served as the only light in the experimental chamber. In order to obtain a measure of general motor activity, each restraint chair was suspended from a wooden enclosure in a way that allowed the chair to swing in any lateral direction when the animal moved. This movement was recorded by two brass rings which were connected to the chair and the fixed wooden enclosure, respectively. These brass rings acted as electrical contacts which served to trigger an electronic counting device. A more detailed description of this measure has been presented previously (Love & Houser, 1973). To insure that extraneous sounds did not disrupt the animal's performance, white noise (90 dB) was presented via a speaker mounted inside the sound-attenuated enclosure. Standard scheduling and recording equipment was located in an adjacent room.

Procedure

Initial training. All six animals were initially trained to press the response lever to escape shock by a method of autoshaping. Animals were placed in the restraining chair, and electrodes were fastened to the tail. The behavioral schedule commenced upon closure of the environmental-chamber door and onset of the houselight. The schedule consisted of a Sidman nondiscriminated avoidance procedure with a response-shock (R-S) and a shock-shock (S-S) interval of 20 sec. The shock intensity was placed at 3 mA for a maximum duration of 5.0 sec. Animals could escape the shock by simply depressing the lever located in the restraint chair, thus terminating the shock and postponing onset of the next shock for 20 sec. Every leverpress response made during the R-S interval postponed shock for another 20 sec. All animals quickly learned to escape and then avoid these shocks by making the appropriate leverpress response within the first few sessions. After 10 2-h sessions (i.e., 20 h), the shock duration was decreased to a maximum of .8 sec, while the intensity was increased to 5 mA for the remainder of the experimental period.

Animals were then subjected to 30 2-h daily sessions (i.e.,

60 h) 5 days per week, to establish stable avoidance baselines. After this training, CS habituation trials were given during five consecutive 2-h daily sessions. Under these conditions, five 3-min CSs were presented at 18-min intervals throughout the session. The CS consisted of dimming the houselight by connecting a 100-ohm resistor in series with the 28-V houselight. After five sessions, no change in response rate was noted during the CS for any of the animals. At this point, unavoidable shocks were programmed to occur simultaneously with offset of each of the five CSs presented during each daily session. These unavoidable shocks were of the same intensity and duration as the avoidable shocks (i.e., 5 mA-.8 sec). Animals were then subjected to 50 sessions (100 h) under this schedule to insure stable baseline performance before drug trials were initiated.

Drug trials. All drug or control solutions were administered orally in a volume of 5.0 ml/kg. Oral administration consisted of placing an intubation needle down the throat and discharging the liquid solution directly into the animal's stomach immediately before the initiation of the testing procedure. The drug (Δ^9 -THC) was supplied in 5-ml ampules which contained 25 mg of Δ^9 -THC dissolved in a 95% alcohol solution. The drug was stored under refrigeration at all times in a sealed vial to prevent evaporation of the alcohol vehicle. Preparation of stock solutions of Δ^9 -THC consisted of mixing 15 ml of the drug with 78.75 ml of sugared orange juice. This solution, when administered in a volume of 5.0 ml/kg, represented a dosage of 4.0 mg/kg. Higher dosages were prepared by evaporating the alcohol in the drug solution so that no more than 15 ml of 95% alcohol was mixed with the 78.75 ml of orange juice. Thus, the concentration of alcohol administered with the various dosages of Δ^9 -THC was held constant. Control solutions consisted of orange juice or 15 ml of 95% alcohol mixed with 78.75 ml of orange juice administered in a volume of 5.0 ml/kg.

Animals were administered the various drug or control solutions on three consecutive daily sessions which were conducted at the same time each day. The six subjects each received three control sessions (orange juice only), followed by three sessions under the alcohol control solution (15.2%) prior to each 3-day drug-dosage period. The dosage of Δ^9 -THC in the first drug period was 4.0 mg/kg for all subjects, 12.0 mg/kg for all subjects in the second period, and 8.0 mg/kg in the third period. A final 3-day control period (orange juice only) completed the series of 30 consecutive daily sessions over which the drug dosages were administered.

Behavioral measures. The behavioral measures taken during this experiment consisted of the total number of leverpress responses made during the 2-h daily sessions, as well as the total number of shocks received. In addition, the cumulative number of responses made during the 3 min preceding onset of each CS were recorded, along with the cumulative number of responses emitted during the five daily CS presentations, on two separate electronic counters. These two measures made it possible to determine if the CS affected response rate by simply comparing the rates emitted immediately before CS onset with rates during the CS. These comparisons were made by computing a ratio first described by Annau and Kamin (1961). The ratio is $B/A + B$, where B represents the number of avoidance responses emitted during the CS, and A the number of avoidance responses during the 3 min before CS onset. This ratio has limits of .00 (representing total suppression during the CS) and 1.00 (representing no responses before the CS with some occurring during the CS). A ratio of .50 would indicate no change in rate during the CS; ratios below this represent suppression, and ratios above .50 represent facilitation. Finally, a measure of general motor activity was obtained from each animal during the 2-h experimental sessions. In addition to a total measure of daily activity, measurements of activity 3 min before and during the CS were recorded on two separate counters. These measures

Table 1
Source of Variance, Degrees of Freedom, and F Values for Analyses of Variance Performed on the Various Behavioral Measures

Source	df	Response Rate	Shock Rate	F Value for		
				Facilitation Ratio	Activity	Activity Ratio
A	9	2.93**	2.40*	3.97***	2.87**	.76
B	2	1.03	1.50	.72	.96	.62
S	5					
AB	18	.63	1.07	.96	1.11	1.29
AS	45					
BS	10					
ABS	90					
Total	179					

Note—A = drug or control treatment, B = days, S = subjects.
* $p < .05$ ** $p < .025$ *** $p < .005$

made it possible to compute ratios identical to those outlined above to indicate whether activity increased or decreased during the presentation of the aversive CS.

RESULTS

Table 1 presents the results of the two-factor (within) analyses of variance (Myers, 1966) performed on the various behavioral measures taken during the study. As can be seen from the table, there were significant treatment effects in the response rate, shock rate, activity, and facilitation-ratio data. Multiple comparisons (i.e., F tests 1/45 df, Myers, 1966) were then carried out between the three orange juice control periods and three alcohol control periods for each of the four above measures to see if the alcohol solution alone influenced the behavioral measures. None of these tests proved to be significant for any of the measures. Thus, the alcohol solution did not appear to affect any of the behavioral data. Finally, three similar comparisons were made between each orange juice control period and the succeeding dosage series of Δ^9 -THC for all of the above measures. The results of these tests will be discussed in detail below.

Figure 1 presents the mean number of leverpress responses and mean number of shocks for the six squirrel monkeys during the 30-day experimental period. This figure indicates that Δ^9 -THC was able to reduce the mean number of avoidance responses emitted and augment the mean number of shocks received under the 4.0 and 12.0 mg/kg dosages. The three F tests performed on the data, however, indicated that only the highest dosage of Δ^9 -THC significantly ($p < .05$) reduced response rate ($F = 4.32$, $df = 1/45$) and elevated the number of shocks received ($F = 5.14$, $df = 1/45$) when compared to the preceding control (i.e., orange juice only) series. As was mentioned above, the alcohol control solution had no reliable effects on either measure during the three separate occasions it was administered.

Figure 2 presents the mean response-facilitation ratios

for the six animals subjected to the various dosages of Δ^9 -THC. The data in Figure 2 indicate that the animals normally demonstrated acceleration of response rate during the aversive CS, since the mean ratios remained considerably above .50. The results of the three comparisons (i.e., F tests) indicated that the vehicle or alcohol control solution, by itself, had no effect on the facilitation ratios during the three separate occasions it was administered. On the other hand, Δ^9 -THC significantly lowered the mean facilitation ratios below that noted under control (i.e., orange juice) conditions under all dosages tested. Furthermore, a rough dose-response curve was generated with the two higher dosages (i.e., 12.0, 8.0 mg/kg) reducing these ratios ($F = 6.48$; $df = 1/45$; $F = 8.03$, $df = 1/45$; $p < .025$) to a slightly greater degree than the lowest dosage ($F = 4.36$, $df = 1/45$; $p < .05$). No signs of drug tolerance were noted in the facilitation-ratio data of Figure 2 within

Figure 1. Mean number of leverpress responses in thousands and mean number of shocks received for six squirrel monkeys subjected to orally administered control solutions of orange juice (C), alcohol and orange juice (ALC) or various dosages of Δ^9 -tetrahydrocannabinol (THC) dissolved in a vehicle consisting of alcohol and orange juice. All dosages of THC are given in mg/kg. Neither ordinate is scaled from zero.

Figure 2. Mean response facilitation ratios for six squirrel monkeys subjected to orally administered control solutions of orange juice (C), alcohol and orange juice (ALC) or various dosages of Δ^9 -tetrahydrocannabinol (THC) dissolved in a vehicle consisting of alcohol and orange juice. All dosages of THC are given in mg/kg. The ordinate is scaled from .51 to .67.

dosages although a general depressant effect on the ratios was noted across the 30-day experimental period. Thus, it would appear that Δ^9 -THC was able to differentially reduce avoidance response rates during the aversive CS.

Figure 3 presents the mean session activity rates for the six monkeys subjected to the various dosages of Δ^9 -THC. The data in Figure 3 clearly indicate that all three dosages were able to reduce mean activity during the 2-h session. The results of the three F tests between control and drug-dosage sessions indicated, however, that due to the large amount of between-subject variance caused by a very low baseline activity level in one animal, only the 4.0 and 8.0 mg/kg dosages significantly reduced mean activity ($F = 6.03$, $df = 1/45$; $F = 5.37$, $df = 1/45$). No clear dose-response relationship was apparent in this data, nor were there any signs of drug tolerance within dosage sessions. Finally, an analysis of the activity-facilitation ratios indicated that none of the drug or control solutions reliably affected the increase in activity normally noted during the presentation of the aversive CS. Under control conditions, the mean activity ratios for all six animals was .61; under alcohol control conditions, .53; and under drug conditions, .56. Although the mean drug ratios were below baseline values, the data were extremely variable both between and within animals. Thus, no statistically reliable effects were noted with regard to this measure.

DISCUSSION

The present results indicate that Δ^9 -THC was able to produce decrements in avoidance response rate which led to slightly higher shock rates under the 12.0 mg/kg dosage. These results are in agreement with earlier work which demonstrated that THC was able to retard discriminated avoidance behavior in rats (Pradhan, Baily, & Ghosh, 1972; Webster, Willinsky, Herring, & Walters, 1971). Since only the highest dose (i.e., 12.0 mg/kg) produced significant decrements, it would appear that avoidance response rate in well-trained animals is relatively resistant to the effects of marijuana compounds.

The consistent reduction in the response ratios noted after the administration of Δ^9 -THC are of interest because, unlike overall response rate, these data were sensitive to all doses tested. Administration of Δ^9 -THC produced reliable reductions in response rate during the aversive CS under all three dosages (i.e., 4.0, 8.0, 12.0 mg/kg). Since the enhanced response rates during the aversive CS have been assumed to reflect increased levels of fear (Maier et al., 1967), it is possible that Δ^9 -THC may reduce the level of fear motivation generated by the aversive CS. This interpretation is supported by the work of Gonzalez et al. (1972), who noted that rats demonstrated a reduction in conditioned

Figure 3. Mean activity counts for six squirrel monkeys subjected to orally administered control solutions of orange juice (C) alcohol and orange juice (ALC) or various dosages of Δ^9 -tetrahydrocannabinol (THC) dissolved in a vehicle consisting of alcohol and orange juice. Each bar represents the mean of 3 consecutive days under drug or control solutions. All dosages of THC are given in mg/kg. The ordinate begins with a value of 2,500 mean activity counts and thus, is not scaled from zero.

suppression and defecation during an aversive CS after treatment with marijuana extract. It thus appears that the tetrahydrocannabinols may have antianxiety properties in squirrel monkeys as well as in rodents.

The overall activity data indicated that Δ^9 -THC was able to produce considerable signs of sedation and that this effect demonstrated a lack of drug tolerance. Other reports (Hardman, Domino, & Seevers, 1971; Martinez, Stadnicki, & Schaeppi, 1972) have indicated that Δ^9 -THC and other synthetic tetrahydrocannabinols are able to produce considerable signs of behavioral depression in monkeys. In contrast to the present results, however, Hardman et al. (1971) have reported that the behavioral depressive effects of the tetrahydrocannabinols do demonstrate tolerance in the monkey after 7 days of drug treatment. Since none of the dosages given in the present study were administered for more than 3 consecutive days, it is possible that continued administration of Δ^9 -THC might have produced some sign of drug tolerance in the activity data.

The facilitation ratios obtained by comparing the activity rates before and during the aversive CS indicated that squirrel monkeys normally demonstrate increased activity during an aversive CS. Thus, the augmented avoidance response rates during the CS are correlated with increased spontaneous motor activity. Administration of Δ^9 -THC, however, consistently affected only the response-rate facilitation noted during the CS without changing the relative amount of activity generated during this period. If Δ^9 -THC does reduce response rate during the CS by reducing level of fear motivation, it may be that response rate more accurately reflects level of fear motivation than do changes in

activity.

Finally, the fact that Δ^9 -THC was able to affect some behavioral measures (i.e., response-facilitation ratios and general motor activity) in all doses, while affecting others (i.e., response and shock rate) only under the highest dose, highlights the desirability of obtaining more than one behavioral measure when one is attempting to assess the effects of pharmaceutical agents. It is apparent from the present data that the response-facilitation-ratio measure is more sensitive to the effects of Δ^9 -THC than the simple measure of overall response rate.

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REFERENCE NOTE

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