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Effects of Δ^9-tetrahydrocannabinol (THC) on conditioned avoidance responding in mice and rats and the one-trial conflict test in rats

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When administered orally to mice, 100.0 mg/kg of Δ^9-tetrahydrocannabinol (THC) produced an overall increase in avoidance performance. However, a lower dose, 50.0 mg/kg, demonstrated a slight but nonsignificant ($p > .4$) decrease in the avoidance performance of mice. In rats, following oral administration, THC produced an increase at 25.0 mg/kg and a decrease at 100.0 mg/kg in avoidance performance. On the other hand, 8.0 mg/kg i.p. of THC produced an increase in the mean number of shocks taken by rats in the one-trial conflict test. This increase was similar to the one observed for diazepam at 2.5 mg/kg. When evaluated with respect to neurotoxic activity as measured in the rotarod test, these data are suggestive of some "disinhibition" activity for THC.

The depressant activity of Δ^9-tetrahydrocannabinol (THC), the major psychoactive constituent of marihuana (Mechoulam, 1970), has been well established using a number of operant behavioral paradigms in a variety of laboratory animal species including rats (Grunfeld & Edery, 1969; Karniol & Carlini, 1973), monkeys (Conrad, Elsmore, & Sodetz, 1972; Ferraro & Billings, 1972; Scheckel, Boff, Dahlen, & Smart, 1968), and pigeons (Frankenheim, McMillan, & Harris, 1971; Kosersky, McMillan, & Harris, 1974). In addition, the comprehensive antiaggressive activity of THC has been evaluated (Dubinski, Robichaud, & Goldberg, 1973), confirming its inhibitory action on isolation-induced aggression in mice, electroshock-induced fighting in mice and rats, mouse-killing behavior of rats, and increased excitability and aggressivity in rats brought on by bilateral septal lesions. Moreover, natural aggression in Chinese hamsters (ten Ham & van Noordwijk, 1973) and monkeys (Conrad et al., 1972; Ferraro & Billings, 1972; Scheckel et al., 1968) has also been inhibited following THC administration. These findings, which are suggestive of potential neuroleptic or "disinhibition" activity, prompted us to explore the effects of THC in tests specifically designed to uncover such actions, that is, the shuttlebox avoidance test and one-trial conflict tests, respectively. Specificity of action was revealed, based on activity at doses below those that cause sedation or impairment of motor performance as measured in the rotarod test for neurotoxicity.

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METHOD

Animals

The experiments in this investigation were conducted using CD-1 male mice (18-26 g) and CD male rats (150-210 g) obtained from Charles River Breeding Laboratories, North Wilmington, Massachusetts. Experiments were begun only after an acclimation period of at least 4 days to the laboratory environment, which consisted of automatically controlled illumination (with 12 h of light alternating with 12 h of dark), regulated temperature at 21°C-23°C, and a relative humidity range of 20%-30%. For the rotarod experiments, mice were housed 10 per cage, whereas rats were housed 8 per cage. Animals used for shuttlebox avoidance and one-trial conflict experiments were housed individually. All animals had access to food and water ad lib, with the exception of 12-18 h prior to oral administration of THC or if otherwise specified.

Drugs

A stock solution consisting of THC solubilized in 100% propylene glycol at a concentration of 100 mg/ml was used for preparation of all drug dilutions. In mice, the required dosages of THC were suspended immediately before use in a 1.0% Tween 80-isotonic saline solution to a final propylene glycol concentration of 10%, as previously reported (Sofia, Kubena, & Barry, 1973). THC was administered to mice orally in a volume of .1 ml/10 g of body weight. In rats, the required doses of THC were prepared in 100% propylene glycol solution and administered either orally or intraperitoneally in a volume of .1 ml/100 g of body weight.

Rotarod Test

Neurotoxicity was measured in rodents according to the method of Dunham and Miya (1957). Animals were trained to maintain their balance for at least 1 min on a rod 3.2 cm in diameter and rotating approximately 5 rpm. Animals were evaluated for their ability to maintain their balance on the rotating rod 30 min after THC administration. The number of animals that fell from the rotating rod more than once during a 1-min trial period was recorded for each group tested. The neurotoxic $dose_{50}$ (NTD_{50}) (i.e., the dose that effectively caused 50% of the animals to fall from the rotating rod) was calculated according to the method of Litchfield and Wilcoxon (1949).

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Shuttlebox Avoidance Test

Evaluation was made of the rate at which mice or rats will perform an avoidance response to shock by shuttling back and forth in a two-compartment shuttlebox (Bovet, Bovet-Nitti, & Oliverio, 1969). The mouse shuttlebox (24 x 9.5 x 13 cm) was divided into two compartments by a 4-cm-high electrifiable hurdle, and the rat shuttlebox (45 x 19.5 x 20 cm) was divided by a 6-cm-high electrifiable hurdle. Both shuttleboxes were enclosed in ventilated sound attenuated chambers.

Test sessions consisted of 60 trials daily. Each trial consisted of a 5-sec conditioned stimulus (CS) of tone and light in the unoccupied chamber followed by a 10-sec unconditioned stimulus (UCS; 3.0-mA scrambled shock), during which the tone and light remained on in the unoccupied chamber until a response was made. This was followed by a 45-sec intertrial interval (ITI), during which no tone or light was present. A response (hurdle cross) during the CS was designated an avoidance response and postponed the UCS for that particular trial. A response made during the UCS was designated an escape response and terminated the shock for that particular trial. If no response was made, the subject was considered "incapable" of shuttling back and forth, and this was recorded as a blocked response. Responses made during the ITI were recorded as intertrial responses and had no effect on either the CS or the UCS.

Untreated animals underwent one pretraining session on the day prior to experimentation. The following day, these animals were assigned to either a vehicle control or a drug treatment group according to their respective pretraining performance levels. Performance levels were evaluated 30 min after THC administration for a 60-trial session. Mean and standard errors were calculated, and significance was determined using Student's t test. Percent change from the vehicle control group's performance levels for total, intertrial, avoidance, escape, and latency-to-escape responses was calculated according to the following formula: [(drug – control)/control] × 100 = percent change.

One-Trial Conflict Test

The method of Vogel, Beer, and Clody (1971) was used to evaluate the "disinhibition" properties of THC in rats. The apparatus consisted of a small environmental cubicle (30.0 x 24.5 x 27.0 cm) that contained a stainless steel grid floor and a water bottle with a metal sipping tube at one end. The sipping tube was recessed 1.0 cm in an insulated black plastic cap, and a drinkometer circuit was connected between the drinking tube and the grid floor so that the subject completed the circuit from the drinking tube to the grid floor. Naive rats were water deprived 48 h prior to testing. Animals were individually placed in the apparatus and allowed to sip water from the drinking tube. Scrambled shock (2.0 mA for 1.0 sec duration) was delivered following either 20 responses (sips) or five holds (i.e., whenever the subject locked up the drinkometer circuit for at least 2.0 sec by extending the entire mouth over the sipping tube).

Subjects were placed in the apparatus 60 min after intraperitoneal administration of THC because of the shortened test trial period, and they were allowed 2 min to locate the sipping tube. A 3-min test trial period was initiated at the conclusion of the first shock, and subsequent shocks were delivered after each 20 licks or five holds throughout the entire session. The number of shocks delivered was recorded for each subject, and statistical comparisons of the mean number of shocks taken were calculated by the Mann-Whitney U test (Siegel, 1956, Chapter 6).

RESULTS

Rotarod Test

The neurotoxic effects of THC in mice and rats are presented in Table 1. No oral NTD_{50} value could be determined up to the maximum dose tested in mice (80 mg/kg). In contrast, neurotoxic effects were demonstrated in rats with a calculated oral NTD_{50} value and 95% confidence limits of 51 mg/kg (29.9-87.0).

Shuttlebox Avoidance Test

The results of oral administration of THC to mice and rats on overall shuttlebox avoidance performance are presented in Table 2. Figures 1 and 2 depict avoidance performance in blocks of five trials over the entire 60-trial session for mice and rats, respectively.

In mice, an increase in overall avoidance performance, with a concomitant decrease in escape responses was apparent with THC at 100 mg/kg (Figure 1). Moreover, Figure 1 depicts an increase in avoidance performance above control values in all but three blocks of five trials for THC at 100 mg/kg. THC, 50 mg/kg, demonstrated a slight reduction (17%) in avoidance performance, with a concomitant increase (18%) in escape response. However, effects at this dose (50 mg/kg) did not differ significantly from control values.

In rats, 25 mg/kg of THC exhibited a marked increase in overall avoidance performance, with a concomitant decrease (43%) in escape responses. Conversely, THC at 100 mg/kg demonstrated a reduction (28%) in overall

Table 1
Neurotoxic Effects of Δ⁹-Tetrahydrocannabinol in Rodents

	Mouse	Rat
Oral Dose (mg/kg)	20.0	5.0
	40.0	10.0
	80.0	20.0
		40.0
		80.0
Number of Animals Tested	30	48
NTD_{50}*	> 80	51.0†

*±95% confidence limits. †29.9-87.0.

Table 2
Effects of Δ⁹-Tetrahydrocannabinol on Shuttlebox Avoidance Performance in Rodents

Species	Oral Dose (mg/kg)	Number of Animals Tested	Percent Change from Control					Mean Change*
			Total Responses	Intertrial Responses	Avoidance Responses	Escape Responses	Latency to Escape	
Mouse	50.0	8	−19.6	−71.3	−16.7	18.2	71.2	.12
	100.0	8	11.3	69.8	30.0	−22.8	9.8	−.02
Rat	25.0	8	11.8	342.5	45.9	−43.3	−58.3	.00
	100.0	8	.0	−21.0	−27.9	43.3	119.6	1.13

*Mean change in number of blocked responses.

avoidance performance, with a concomitant increase (43%) in escape responses. Graphical representation of these effects (25 and 100 mg/kg) clearly demonstrates an increase and a decrease, respectively, in avoidance performance, compared with controls (Figure 2).

One-Trial Conflict Test

In an attempt to further elucidate the potential disinhibitive property of THC, a one-trial conflict test was used. THC was administered intraperitoneally instead of orally in this test to enhance the possibility of obtaining the desired effect (i.e., disinhibition). Figure 3 depicts the effects of THC in rats at 60 min posttreatment. Diazepam, 2.5 mg/kg, was used as the positive reference standard. Significant ($p < .01$) disinhibition was demonstrated with THC at 8 mg/kg. THC at 12 mg/kg suppressed the number of responses made in some animals while causing others not to respond at all. Upon observation, those animals that failed to respond also demonstrated catatonia.

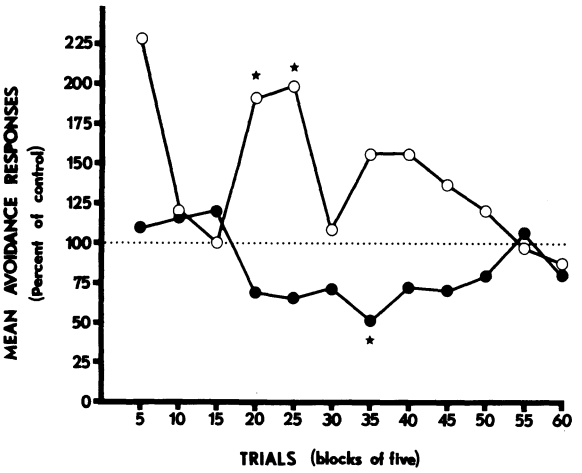

Figure 1. Effect of Δ^9-tetrahydrocannabinol (THC) on avoidance performance in mice. Filled circles indicate THC 50 mg/kg; open circles indicate THC 100 mg/kg; an asterisk indicates significance at $p < .05$ (Student's t test).

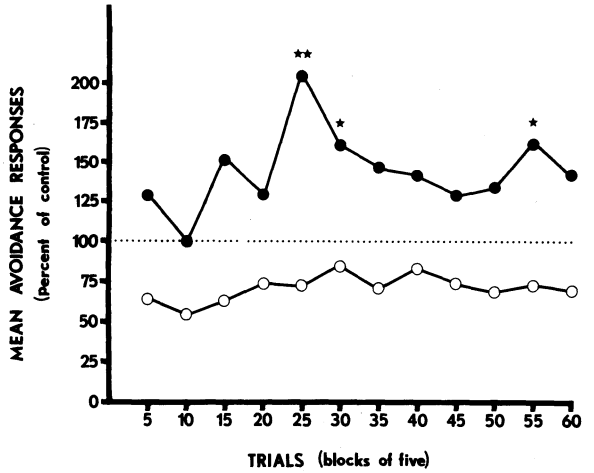

Figure 2. Effect of Δ^9-tetrahydrocannabinol (THC) on avoidance performance in rats. Filled circles indicate THC 25 mg/kg; open circles indicate THC 100 mg/kg; a single asterisk indicates significance at $p < .05$, and a double asterisk indicates significance at $p < .01$ (Student's t tests).

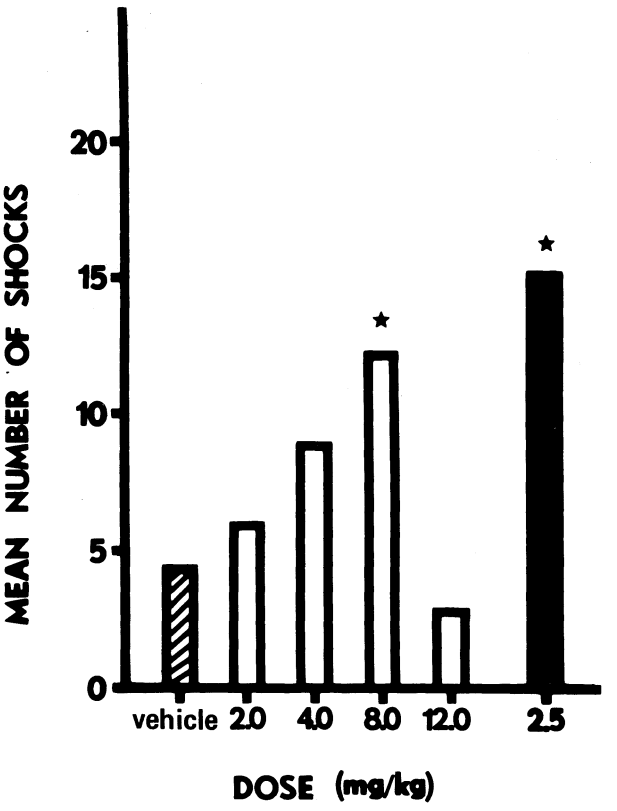

Figure 3. Effects of Δ^9-tetrahydrocannabinol (THC) given intraperitoneally to rats and tested in a one-trial conflict schedule at 60 min posttreatment. Shaded squares indicate vehicle control; open squares indicate THC; closed squares indicate diazepam; an asterisk indicates significance at $p < .01$ (two-tailed Mann-Whitney U test).

DISCUSSION

Although no neuroleptic activity was demonstrated for THC in mice, it is interesting to note that the lower dose produced a slight reduction in avoidance performance, while the higher dose produced an enhancement of avoidance performance. Scheckel et al. (1968) reported similar results for monkey barpressing: At lower doses the monkeys demonstrated reduced rate of continuous avoidance, whereas at higher doses an enhancement was observed, presumably due to a stimulating effect of THC. These authors also noted behavioral changes, with depression beginning approximately 3 h posttreatment in the higher dose groups. In some animals death resulted following severe depression. In our experiment, it is possible that mice administered the higher dose of THC underwent a similar course of drug effect because slight to mild depression was noted following removal from the shuttlebox test chamber (90 min posttreatment); however, no deaths were observed.

In the rat shuttlebox experiments, disruption of the learned behavior by THC was observed at the high dose (100 mg/kg) but not at the low dose (25 mg/kg). These results were interpreted as "disinhibitive" since enhanced shuttlebox avoidance performance was observed at doses below the NTD_{50} and reduced shuttlebox avoidance performance was observed at doses above the

NTD_{50} for THC. This profile of activity is generally indicative of the anxiolytics.

Further indication of possible disinhibition activity of THC in rats was demonstrated in the one-trial conflict test. Preliminary studies following a 30-min absorption period demonstrated some slight activity for THC. However, following a 60-min absorption period, THC at 8 mg/kg demonstrated a significant increase in the mean number of shocks taken, similar to the increase recorded for the positive reference standard (diazepam, 2.5 mg/kg). Increasing the dose of THC above 8 mg/kg resulted in a reduction of the mean number of shocks taken and general overt depression. These results coincide well with the intraperitoneal results for rotarod and shuttlebox performance reported by Grunfeld and Edery (1969). In that study, a 100% decrease in shuttlebox performance observed at 20 mg/kg was attributed to the depressive effects of THC. Thus, within a narrow dose range, THC apparently demonstrated some disinhibition properties in the rat one-trial conflict test.

Conrad et al. (1972) have demonstrated that high doses of THC are generally needed to produce behavioral effects in the chimpanzee following oral administration. Our results are in partial agreement with this, in that high oral doses were required to produce the behavioral effects observed in our tests. In addition, other investigators have shown that THC develops a slow onset of action in mice and rats (Dubinsky et al., 1973; Frankenheim et al., 1971). The one-trial conflict data attest to these findings and suggest the need for further testing by the intraperitoneal route following longer periods for absorption to more clearly define the possible disinhibition activity of THC.

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