Differences in Behavioral Responses of Male and Female Rats to Marijuana[1] (36627)

R. A. COHN, E. BARRATT, AND J. H. PIRCH
(Introduced by J. G. Hilton)

Department of Pharmacology and Toxicology and Department of Neurology and Psychiatry, University of Texas Medical Branch, Galveston, Texas 77550

Delta-9-tetrahydrocannabinol (Δ^9-THC) has been shown to be the major psychotomimetically active constituent in marijuana (1–3). Ataxia, catalepsy, and hyperreactivity to external stimuli have been commonly observed in the dog (4, 5), rat (6, 7), mouse (5, 8), and cat (9, 10) in response to Δ^9-THC or marijuana. In rhesus monkeys Δ^9-THC has been shown to cause stimulation and depression as well as loss of ability or motivation to perform complex memory and visual discrimination tasks (11). Humans treated with marijuana (12) or Δ^9-THC (13) experience euphoria, altered time sense, visual distortions, feelings of depersonalization, and difficulty in concentrating.

The present study concerns the development of a rating scale based on the observed gross behavior of marijuana-treated rats. The rating scale demonstrated the existence of a dose–effect relationship as well as a sex difference in the responses of rats to varying doses marijuana.

Methods. Male and female rats were tested in a series of behavioral tasks which were conducted as follows: (a) "Platform," the animal was placed on a cardboard box 12 in. long, 8 in. wide, and 3 in. high. The amount of time an animal remained on the platform, until he touched any of his paws to the surface of the table, was recorded up to a maximum of 3 min. Placebo-treated animals generally climbed off the platform within the first few seconds. Marijuana-treated animals remained on the platform significantly longer; their behavior was characterized by backward circling, freezing, staring, and marked urination. Although they frequently looked over the edge of the platform, they generally made no attempt to climb off. (b) "Low bar," the animal's front paws were placed on the center of a metal bar 5/8 in. in diameter, 19 in. long, and elevated 3.5 in. from the surface of a table. The animal was timed until he touched either of his front paws to the surface of the table or remained on the bar a maximum of 3 min. Whereas placebo-treated animals invariably got off the bar immeditely, marijuana-treated animals maintained a firm hold on the bar. (c) "Drop," the animal was raised to an height of 12 in. above the surface of a table and dropped. From the time the animal landed on the table to the time he moved either of his front paws was recorded to a maximum of 3 min. Placebo-treated animals moved away upon landing on the table; marijuana-treated animals remained stationary, in the position in which they landed. This effect was seen most consistently with higher doses of marijuana. (d) "Vocalization," the "platform," the "low bar," and the "drop" tests all entailed handling of the animals by the experimenter. The presence or absence of vocalization during each of the previous tests was

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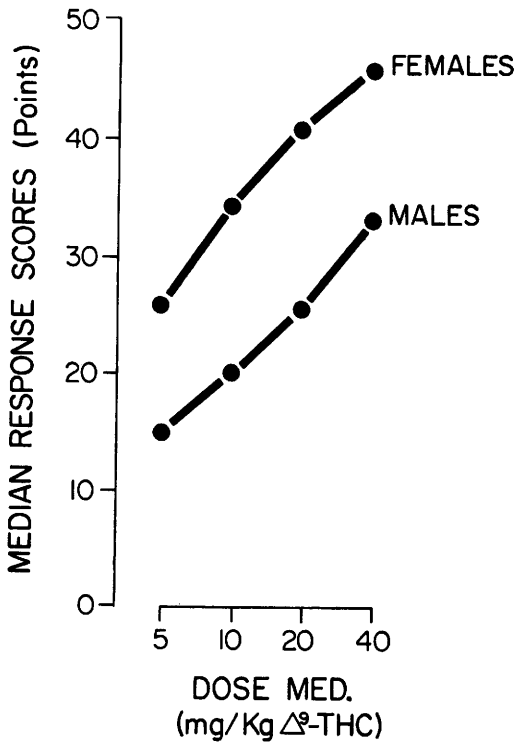

FIG. 1. Median behavior scores of males and females at varying doses of Δ^9-THC.

recorded. Vocalization was one of the most consistent effects seen in marijuana-treated rats; placebo-treated animals rarely vocalized in response to handling. (e) "Dowel," a standard "lead" pencil, 1/4 in. in diameter and 7 in. long, was slowly moved forward from a distance of 12 in. to the tip of the animal's nose. The procedure was repeated 9 additional times in succession. The number of times an animal remained immobile in response to presentation of the dowel was recorded. Placebo-treated animals responded to the pencil by moving away. Marijuana-treated animals remained stationary and stared at the "dowel"; frequently, they displayed piloerection, vocalization, or aggressive biting. (f) "High bar," the animal was placed on a metal bar 3/8 in. in diameter, 19 in. long, and elevated 4 ft from the floor. The amount of time the animal remained on the bar was recorded to a maximum of 3 min. Whereas placebo-treated animals dropped or jumped off shortly after being placed on the bar, marijuana-treated animals remained suspended from the bar.

Marijuana extract distillate (MED) containing 17.1% Δ^9-THC, 1.7% cannabidiol, 5.4% cannabinol, and other undetermined cannabinoids (kindly supplied by Dr. Scigliano, NIMH) was diluted to a concentration of 20 mg/ml Δ^9-THC with 6% Tween 80 in distilled water. The diluted suspension was administered orally to 42 male and 42 female albino rats (Sprague-Dawley descent, Texas Inbred Mice Co.) in doses of Δ^9-THC of 5, 10, 20, and 40 mg/kg. An additional 4 males and 4 females, the controls, received only the suspending medium in a dose of 1 ml/kg.

One male and one female were randomly chosen and administered the diluted marijuana extract. Dosing was staggered to allow the experimenter to begin testing each animal 3 hr after receiving drug. Each animal was run sequentially through platform, low bar, drop, vocalization, dowel, and high bar and replaced in its cage while its sex pair was sequentially run through the behavioral tasks. This procedure was repeated an additional 3 times so that each animal was run through the entire sequence a total of 4 times. The order of presentation of the tasks was never varied. The results were quantified as follows: (a) In all timed tasks (platform, low bar, drop, and high bar) animals received 1/4 point for every 15 sec they performed the particular response up to a maximum of 3 min. (b) "Vocalization" was scored on the basis of 1 point for each of 3 tasks (platform, low bar, and drop) in which the animal squealed in response to handling by the experimenter. (c) The dowel was scored on an all-or-none basis. If an animal remained in place for 7 out of 10 presentations of the pencil he received 3 points; otherwise, he received zero points. Thus, an animal could receive from zero to 3 points for each of the 6 tasks in each of the 4 trials. The sum of the scores of the individual tasks constituted the behavior rating score. A maximum of 72 points could be attained for each animal; higher scores indicated greater drug effect.

Results and Discussion. Median response scores of both male and female rats treated

TABLE I. Median Behavioral Scores of Control and MED-Treated Rats.

Dose Δ^9-THC (mg/kg)	Males	Females	Mann-Whitney U test			
			N_1	N_2	U	p
0	2.13	1.50	4	4	6.5	>.3
5	15.13[a]	26.00[b]	10	10	19	.02
10	20.25	34.50	12	12	28	<.02
20	25.50	41.00	9	9	14	.02
40	33.25[c]	46.00[d]	11	11	28	<.05

[a] A U test between control males and males at 5 mg/kg Δ^9-THC was significant at the .02 level ($U = 1$, $N_1 = 4$, $N_2 = 10$).

[b] A U test between control females and females at 5 mg/kg Δ^9-THC was significant at the .002 level ($U = 0$, $N_1 = 4$, $N_2 = 10$).

[c] A U test between males at 5 mg/kg Δ^9-THC and males at 40 mg/kg Δ^9-THC was significant at the .02 level ($U = 19$, $N_1 = 10$, $N_2 = 11$).

[d] A U test between females at 5 mg/kg Δ^9-THC and females at 40 mg/kg Δ^9-THC was significant at the .002 level ($U = 2$, $N_1 = 10$, $N_2 = 11$).

with marijuana followed a log dose–response relationship; increasing scores reflected increased levels of intoxication (Fig. 1). At doses of Δ^9-THC of 5, 10, 20, and 40 mg/kg, median response scores were consistently higher for females than males. In fact, the median score for males at 20 mg/kg Δ^9-THC was comparable to the score for females at 5 mg/kg Δ^9-THC; likewise, the median score for males at 40 mg/kg Δ^9-THC was comparable to the score for females at 10 mg/kg Δ^9-THC. A comparison of the scores of males and females by way of a two-tailed Mann-Whitney U test showed that females had significantly higher scores than males at each dose of marijuana (Table I). Responses of male and female controls, treated with placebo, did not differ significantly from one another. A comparison between controls and animals treated with 5 mg/kg Δ^9-THC was significant for both males and females, demonstrating that the rating scale was capable of differentiating between placebo and a low dose of marijuana. In addition, a U test between scores of animals treated with 5 mg/kg Δ^9-THC and those of animals treated with 40 mg/kg Δ^9-THC was significant for both males and females, indicating that the rating scale was also capable of differentiating between a low dose and an high dose of marijuana.

Since animals were tested in the behavioral tasks 3 hr after receiving marijuana, it was possible that the sex difference in response was due to differences in onset or duration of action. In order to examine this possibility, experiments were conducted to examine the time course of the drug effect. Male and female rats were paired and treated with marijuana extract in a dose of Δ^9-THC of 20 mg/kg. Either 15 min, 1 hr, 2 hr, or 7 hr

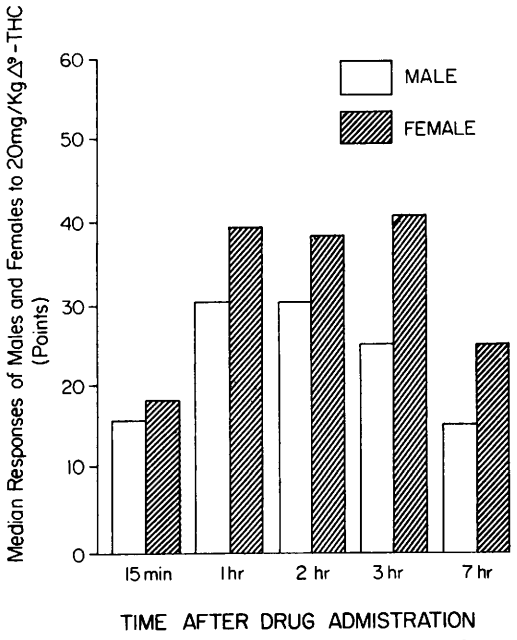

FIG. 2. Median responses of males and females at various time periods after administration of 20 mg/kg Δ^9-THC.

TABLE II. Median Responses to 20 mg/kg Δ^9-THC at Various Time Periods After Administration.

Time after drug[a]	Males	Females	N_1	N_2	U	p
15 min	16.25	18.38	10	10	31.5	>.10
1 hr	30.88	39.63	10	10	8	<.002
2 hr	30.75	38.63	10	10	23	<.05
3 hr	25.50	41.00	9	9	14	.02
7 hr	15.63	25.25	10	10	23	<.05

[a] Time at which testing procedure was initiated.

after receiving drug, animals were tested in the series of behavioral tasks.

Median responses were higher for females than males at all time intervals after drug administration (Fig. 2). Both males and females tested 1, 2, or 3 hr after receiving drug had higher median scores than animals tested 15 min or 7 hr after receiving drug. The time course of the behavioral effects appears to correlate with the work of other investigators (14) who found that brain radioactivity after administration of ^{3}H-Δ^1-THC was higher at 1, 2, and 4 hr than at 20 min and 6 hr. Since we also found a significant sex difference at 1, 2, 3 and 7 hr (Table II), it would be of value to compare brain levels of labeled Δ^9-THC in males and females at these time periods.

The sex differences to marijuana extract were reflected in the behavior response scores. The fact that females had significantly higher scores than males does not necessarily mean that they experienced a greater overall effect. It is indeed possible that the quality of effect, rather than the magnitude, is different for males than females; the behavioral tasks to which the animals were subjected may have been biased toward accentuating the female drug response or minimizing the male response.

On the other hand, if the sex differences are truly quantitative in nature, then one must look at possible differences in biotransformation, distribution, or tissue sensitivity to marijuana. Future research will be aimed at elucidating the nature of this sex difference.

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