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Failure to Obtain "Cannabis-Directed Behavior" and Abstinence Syndrome in Rats Chronically Treated with Cannabis Sativa Extracts*

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Abstract. Experiments were performed to verify whether chronic treatment with *Cannabis sativa* extracts would induce dependence and/or abstinence symptoms in rats. In Experiment *one*, rats ingested cannabis extract as the only fluid for 126 days. On days 1, 43, 48, 62, 80, 92 and 119 when the animals were in abstinence from previous administration of marihuana for 0 to 96 h, behavioral measures and choice tests between marihuana suspension and control solution were carried out; the rats preferred to drink the control solution and no behavioral symptoms indicating abstinence were noted. In Experiment *two*, rats were trained to drink a marihuana suspension or control solution in order to be rewarded with sweetened milk. After 22 sessions water was substituted for the milk. Extinction curves for both groups were similar, and in choice tests among cannabis suspension, water and control solution, the animals refused to drink the marihuana suspension. In Experiment *three*, rats received i.p. injections of a cannabis extract or control solution during 36 days. From days 37—40 the drugs were withheld and sensitivity to pentylenetetrazol was assessed. Marihuana chronically treated rats were more resistant to convulsions and fewer died after pentylenetetrazol. In Experiment *four*, rats were treated with marihuana extract, control solution or sodium barbitone during 73 days. At days 74—76, when undergoing 24, 48 and 72 h of abstinence, the animals were exposed to sound from a bell. Animals in abstinence from barbitone showed a high incidence of convulsions; marihuana treated rats did not differ from control animals.

These results suggest that rats do not self administer marihuana (Experiments *one* and *two*) and do not present abstinence symptoms after prolonged periods of ingestion of the drug (Experiments 1, 3 and 4).

Key words: Cannabis sativa — Drug Dependence — Abstinence Syndrome — Marihuana — Cannabis-Directed Behavior.

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Several authors have reported that marihuana does not induce either physical dependence or an abstinence syndrome in man (New York Mayor's Committee on Marihuana, 1944; Watt, 1965; Goth, 1970; Snyder, 1971), while others describe symptoms of abstinence in heavy users of strong marihuana preparations (Williams, Himmelsbach, Wikler, Rubble, and Lloyd, 1946; Fraser, 1949; Kielholz and Ladwig, 1970). However, to the best of our knowledge, there are no experimental studies with laboratory animals specifically designed to verify whether marihuana would induce dependence. This point may be of importance as it is known that drugs which induce physical dependence and abstinence syndrome in man also induce such abnormalities in and are self administered by laboratory animals. This has been demonstrated for morphine and other opioid drugs, barbiturates and minor tranquilizers (Schuster and Thompson, 1969).

The present paper describes several experiments carried out with rats to study this point. After this work had started, Kaymakçalan (1972) described physiological and psychological dependence on (–) Δ^9-*trans*-tetrahydrocannabinol in Rhesus monkeys.

Experiment 1

The first experiment was undertaken in order to verify: a) whether rats would show a "directed behavior" for cannabis as has been reported for morphine (Nichols, 1963; Stolerman and Kumar, 1970); b) whether rats would show abstinence symptoms during withdrawal from previous cannabis administration as has been described for morphine (Martin, Wikler, Eades, and Pescor, 1963).

Material and Methods

Twenty female Wistar rats 3 months old were individually housed in wire cages measuring $16 \times 18 \times 30$ cm with food provided *ad libitum*. Ten animals received a suspension of a cannabis resin as their only source of fluid (Carlini and Kramer, 1965). The other 10 rats received control solution (1.5 ml Tween-80/liter of water). The cannabis suspension initially contained 0.1 mg/ml of resin and during the first 18 days the concentration was increased by 0.1 mg/ml every second day up to 1.0 mg/ml, which was given for another 108 days. Gas chromatography of the resin revealed 32.7% of (–) Δ^9-*trans*-tetrahydrocannabinol, 11.2% of cannabidiol and and 39.8% of cannabinol. (We are grateful to Drs. J. R. Valle and G. Aucelio for performing the chemical analysis).

During the 4 months of forced ingestion the animals were submitted to the following measures:

24 h Liquid Intake. It was determined once every week for each group of rats.

Body Weight. Assessed at 15 day intervals.

Choice Tests between Cannabis Suspension and Control Solution. Seven choice tests (Nichols, 1963) were given. Two bottles were offered, one with control solution and the other with marihuana suspension; liquid intake was measured 24 h later. The choice tests were at days 1, 43 and 48 when the animals were not previously

deprived of drugs, and at days 62, 80, 92 and 119 when they were in abstinence from marihuana (control solution substituted for marihuana) for, respectively, 24, 48, 72 and 96 h. As will be seen below, the animals had had the opportunity to learn that eventual abstinence symptoms could be relieved by marihuana intake.

Behavioral Measures in an Open-Field Arena and in the Home Cages. In the morning of days 34—39 when the animals were not deprived of marihuana and then at days 55, 76, 86 and 109, when they were in abstinence for, respectively, 24, 48, 72 and 96 h, defecation, ambulation, rearing and grooming in an open field arena were recorded (Masur, Martz, and Carlini, 1971; Masur, 1972). "Wet dog shakes" (Martin *et al.*, 1963) was also recorded in the open-field. In the afternoon of the same days the rats were observed in their home cages for general activity (any spontaneous motion within a 5 sec period), ptosis, piloerection, irritability (response to a blow of air), aggressiveness (attack and biting a wooden rod introduced in the cage) and defecation (number of fecal boluses eliminated from 2.00 to 5.00 p.m.). With the exception of defecation, the other measures were scored from 0 to 3 points according to the intensity. Immediately after the end of these measures marihuana was again given to the deprived rats.

As the days of these deprivation periods and measures occurred before the corresponding periods selected for the choice tests, the rats had a chance to learn beforehand that the eventual aversive symptoms of abstinence could be relieved by the ingestion of the marihuana offered in one of the bottles during the choice tests.

Organ Weights. After the end of experiment (day 127), 5 rats of each group were killed and brain, heart, kidneys and adrenals were weighed. The remaining rats received control solution *ad libitum* during the next 5 days at the end of which, they also had their organs weighed.

To assess the effect of chronic intake of cannabis on organ weights, 2 other groups of 18 male rats each, 2 months old at the beginning of experiment, were housed in groups of 3—5 per cage and cannabis extract or control solution were given as the only liquid for a period of 84 days. Half of the animals of each group were killed and the organs weighed at day 85 and the remaining rats were sacrificed 6 days later, after drinking control solution for 5 more days.

Results

Liquid Intake and Body Weight Measures. The rats which had only marihuana suspension to drink ingested significantly less liquid than the animals given control solution (Table 1). Taking into consideration the concentration of the suspension (1.0 mg/ml), the percentage of Δ^9-THC present in the resin (32.7 %), the amount of suspension ingested, and the weight of the animals, it was calculated that the rats were self administering about 60—100 mg/kg of a cannabis resin corresponding to about 20—33 mg/kg of Δ^9-THC daily.

Body weight of marihuana-treated animals was significantly below that of control rats (Table 1). This reduction was probably due to the daily reduced intake of liquid because in the choice tests (Table 2), with the chance to drink control solution, they drank it in larger amounts than the control rats. Furthermore, when the marihuana-treated rats were allowed, at the end of the experiment, to freely drink the control solution for 5 days, they significantly gained weight (Table 3).

Table 1. Liquid intake and body weight of rats forced to ingest cannabis extract and control solution

Weeks of treatment	Liquid intake (ml ± SD)		Body weight (g ± SD)	
	Control solution	Marihuana	Control solution	Marihuana
1	24.8 ± 2.9	19.8 ± 3.7*	205.7 ± 17.3	203.9 ± 19.6
2	29.6 ± 5.6	22.1 ± 6.0*	—	—
3	25.0 ± 3.8	18.3 ± 4.7	204.3 ± 16.4	210.1 ± 22.1
4	28.5 ± 6.3	20.5 ± 6.4*	—	—
5	23.4 ± 3.4	18.4 ± 4.0*	210.4 ± 15.5	201.3 ± 20.6
6	24.0 ± 2.4	12.0 ± 5.4*	—	—
7	24.9 ± 2.6	16.4 ± 17.1	217.3 ± 13.6	198.6 ± 15.4*
8	25.4 ± 4.4	19.4 ± 11.7	—	—
9	20.8 ± 7.2	16.9 ± 9.9	221.5 ± 11.7	196.1 ± 22.0*
10	24.7 ± 3.8	15.7 ± 6.3*	—	—
11	25.1 ± 2.9	17.1 ± 10.6*	221.7 ± 8.5	204.2 ± 24.7*
12	24.3 ± 8.4	14.3 ± 5.6*	—	—
13	21.8 ± 3.1	18.3 ± 8.5	218.1 ± 7.9	199.0 ± 21.4*
14	20.0 ± 3.4	19.4 ± 7.6	—	—
15	21.6 ± 2.5	15.6 ± 8.6*	222.1 ± 13.5	196.2 ± 16.8*
16	22.0 ± 3.4	14.5 ± 3.5*	—	—
17	18.8 ± 2.3	15.1 ± 4.1*	225.6 ± 12.5	202.9 ± 20.2*
18	23.5 ± 6.0	12.9 ± 3.8*	—	—

* Differs significantly from respective control group (P at least ≤ 0.05; Student t-test).

Table 2. Liquid intake, during choice tests, of rats submitted previously to forced self-ingestion of control solution and cannabis extract during 4 months

Choice test number	Chronic self-administration		Days of abstinence	Ml of liquid (± SD) ingested during choice tests	
	Liquid ingested	Number of days		Control solution	Marihuana
1	Control sol.	1	0	23.8 ± 4.3	4.7 ± 1.6
	Cannabis	1	0	29.3 ± 8.8*	5.0 ± 1.4
2	Control sol.	43	0	27.8 ± 2.7	7.8 ± 1.4
	Cannabis	43	0	40.3 ± 8.6*	3.3 ± 6.2
3	Control sol.	48	0	22.4 ± 5.4	2.7 ± 2.3
	Cannabis	48	0	39.9 ± 6.6*	3.8 ± 4.9
4	Control sol.	62	1	22.4 ± 3.8	2.7 ± 2.0
	Cannabis	62	1	31.9 ± 8.0*	3.6 ± 2.5
5	Control sol.	80	2	20.3 ± 3.9	3.3 ± 1.7
	Cannabis	80	2	37.3 ± 11.0*	3.6 ± 1.8
6	Control sol.	92	3	21.7 ± 2.6	4.0 ± 4.5
	Cannabis	92	3	35.2 ± 8.5*	3.9 ± 5.3
7	Control sol.	119	4	19.6 ± 3.0	2.8 ± 1.9
	Cannabis	119	4	34.4 ± 11.0*	5.6 ± 10.4

* Differs significantly from respective control (P at least ≤ 0.05; Student t-test).

Table 3. Organ weights of rats which chronically self-administered cannabis extract and control solution

Replicate	Chronic self-administration		Extra days with control solution administration	Number of animals	Weight in grams (± SD) of				
	Liquid ingested	Number of days			Adrenal	Kidneys	Heart	Brain	Total body
1	Control sol.	126	—	5	0.072 ± 0.012	1.775 ± 0.126	0.746 ± 0.052	1.742 ± 0.032	215.7 ± 7.8
	Cannabis	126	—	4	0.056 ± 0.015	1.881 ± 0.095	0.638 ± 0.038*	1.673 ± 0.013*	193.3 ± 13.1*
	Control sol.	126	5	4	0.059 ± 0.007	1.893 ± 0.198	0.740 ± 0.031	1.721 ± 0.049	215.0 ± 13.8
	Cannabis	126	5	6	0.059 ± 0.005	1.873 ± 0.254	0.738 ± 0.077	1.709 ± 0.05	209.2 ± 20.4
2	Control sol.	84	—	9	0.050 ± 0.008	1.382 ± 0.145	0.656 ± 0.067	1.611 ± 0.087	180.6 ± 23.9
	Cannabis	84	—	9	0.043 ± 0.012	1.390 ± 0.174	0.603 ± 0.102	1.605 ± 0.088	157.7 ± 17.7*
	Control sol.	84	5	9	0.050 ± 0.008	1.441 ± 0.152	0.702 ± 0.108	1.670 ± 0.108	199.8 ± 22.6
	Cannabis	84	5	9	0.051 ± 0.006	1.379 ± 0.089	0.698 ± 0.093	1.618 ± 0.078	177.5 ± 10.4*

* Differs significantly from respective control group (P at least ≤ 0.05; Student t-test).

It was not possible to increase the concentration of cannabis above 1.0 mg/ml; a few extra rats refused to drink suspensions of 2.0 mg/ml and lost so much weight in 4 days, that the experiment was discontinued.

Choice Tests. In none of the 7 choice tests did the animals prefer to drink marihuana (Table 2). Thus, even when forced previously to drink marihuana for 4 months (choice test number 7) they drank mostly control solution. It is noteworthy that in all choice tests the marihuana-treated rats drank more control solution than the control animals. This probably happened because these animals ingested less liquid during the periods of forced ingestion of marihuana and were dehydrated; this would also explain their underweight (Table 1).

Behavioral Measures. There was no difference between the 2 groups of rats in the measures performed in the home cages. Thus, at days 34—39, 55, 76, 86 and 109 the scores for general activity, ptosis, piloerection, irritability, aggressiveness and defecation were similar. No difference was found either in all the open-field measures.

Organ Weights. Rats which ingested marihuana for 126 days had significantly lower heart, brain and total body weights than their controls (Table 3; first 2 rows). When control solution was given for 5 extra days, these differences disappeared (Table 3; 3rd and 4th rows) indicating that decrease of water could be responsible for the differences in organ and body weights. Similar results were obtained after 84 days of forced ingestion in a replicate experiment (Table 3; 5th to 8th rows).

Experiment 2

The refusal of rats chronically treated with marihuana to drink it in choice tests (Experiment 1) even when in abstinence from the drug, suggests that withdrawal of marihuana did not induce unpleasant symptoms, or rather that if unpleasant symptoms were present, the taste of marihuana was more unpleasant. Therefore, marihuana could not function as a primary reinforcer to mitigate eventual abstinence symptoms. However, the possibility remained that marihuana could function as a secondary reinforcer. According to Harris, Claghorn, and Schoolar (1968) a drug might serve as a secondary reinforcer when, by pairing it with a primary reinforcer such as food, it could acquire secondary reinforcing properties and be later self administered. The possibility that marihuana may function as a secondary reinforcer to humans has been suggested before (Thompson and Pickens, 1969).

Material and Methods

Twelve female Wistar rats 3 months old, housed 6 to a cage, were trained to lick water for 5 sec from a drinking tube adapted to a Skinner box; reward was 0.02 ml of sweetened milk diluted to 1/3. After 8 daily training sessions of 15 min duration

each, the animals were drinking an average of 3.5 ml of water and were rewarded about 30 times. Water was then replaced by a 0.1 mg/ml cannabis suspension in the drinking tube for 6 animals, and for the remaining 6 rats the control solution was used. A new cannabis extract was used containing 35.5% of Δ^9-THC, 13.4% of cannabinol and 51.1% of cannabidiol. The concentration of the cannabis suspension was progressively increased to 0.5 mg/ml which was offered for another 14 days. In the last 3 days the animals were drinking an average of 2.8 ml of the suspension corresponding to 2.5 mg/kg of Δ^9-THC; control animals were drinking an average of 4.5 ml of the control solution. The average amount of reward was 22 and 37, respectively. In the following 5 days the animals were given 15 min extinction sessions daily (water substituted for milk), and after that they were offered a choice test in their home cages, between 3 bottles containing, respectively, water, control solution and cannabis suspension. The liquid intake was recorded 20 h later each day.

Results

Fig. 1 shows the extinction curves for control and marihuana-treated rats. Both groups achieved extinction equally with the curves showing similar shapes, although control animals drank more than marihuana-rats. Fig. 2 shows that all rats refused to drink marihuana in the choice tests carried out during the extinction phase; it is also seen that the

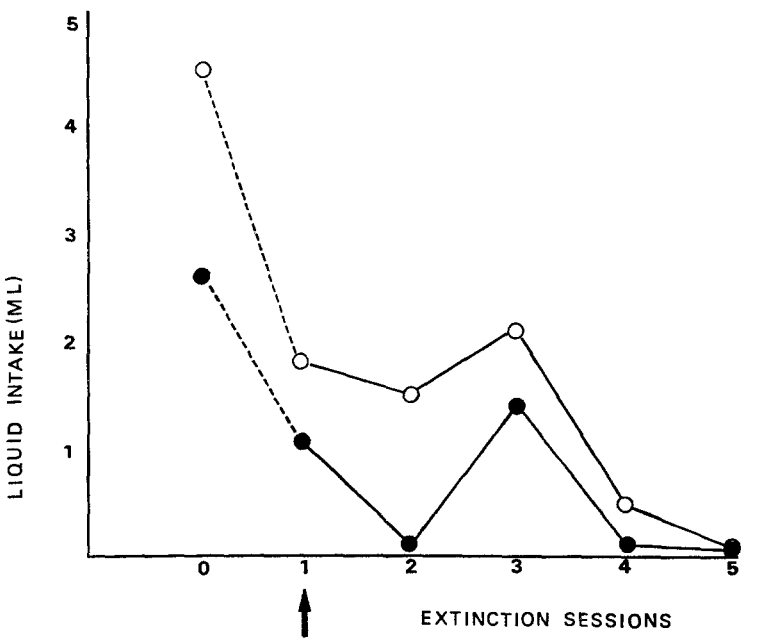

Fig. 1. Extinction curves of rats which self administered a marihuana extract (•——•) and control solution (o——o) in order to be reward with sweetened milk. Session zero (0) indicates the last session with reward. The arrow indicates the first session in which water was substituted for the milk reward

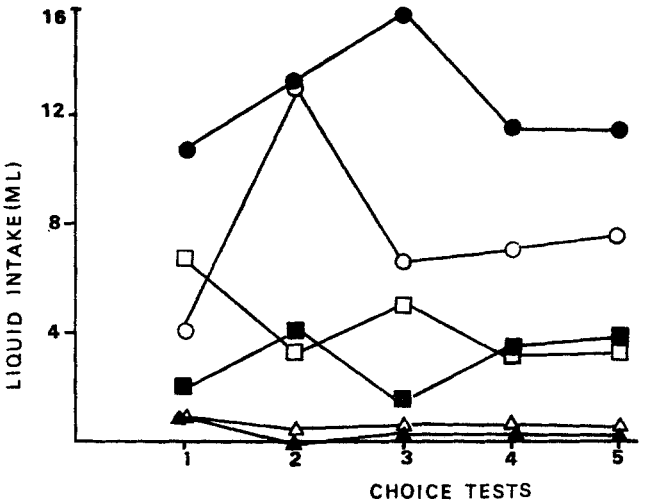

Fig. 2. Total liquid intake in choice tests of 2 groups of 6 rats each, which were previously trained to drink a marihuana suspension (heavy symbols) and control solution (open symbols). Circles, squares and triangles refer, respectively, to intake of water, tween-80 (control solution) and marihuana suspension offered simultaneously in each choice test

animals showed a greater intake of water when compared to the Tween-80 or marihuana suspensions.

Experiments 3 and 4

It was decided to study whether chronic administration of marihuana followed later by drug withdrawal would induce an abstinence syndrome in rats similar to that observed in barbiturate abstinent rats. These animals show evident proneness to develop convulsions either spontaneously or by physical and chemical means; there is also a fall of body weight (Essig, 1967; Stevenson and Turnbull, 1968).

Material and Methods

Experiment 3. Fifty-six Wistar rats of both sexes, 2 months old were housed 5 or 6 to a cage with water and food *ad libitum*. Thirty six of them received 2 i.p. injections, 40 mg/kg each, daily during 36 days, of a new extract of marihuana containing 33.3, 12.2 and 32.7%, respectively, of Δ^9-THC, cannabidiol and cannabinol. Ten other rats were injected i.p. twice daily with the control solution and the remaining 10 animals received i.p. 1.0 ml/kg of 0.9% NaCl solution. From days 37 to 40 the injections were withheld. The sensitivity of the animals to i.p. pentylenetetrazol was assessed in groups of 9 marihuana-treated rats with 24 (day 37), 48 (day 38), 72 (day 39) and 96 (day 40) h of abstinence. Saline and control solution-treated rats were injected i.p. with cardiazol at days 38 and 40, respectively. Sensitivity to cardiazol was measured by a modification of the procedure of Levine,

Payan and Strebel (1963). Briefly, 25 mg/kg of the convulsant were injected every 5 min until death of animals. The maximum dose of cardiazol employed was 125 mg/kg and the time and amount of drug for appearance of the 1st convulsion and of death were recorded.

Experiment 4. The experiment was carried out in 2 phases. In the first, 26 2 month old female rats were given a cannabis extract for 44 days as the only source of fluid. The extract used contained 59.9, 1.4 and 1.03% respectively, of Δ^9-THC, cannabidiol and cannabinol; the concentration of the suspension was raised from 0.1 mg/ml up to 0.6 mg/ml which was given during the last 30 days of the experiment. Simultaneously, 10 other rats ingested a 4 mg/ml barbitone sodium solution and finally 13 rats received control solution. After 1 to 3 days of abstinence from the drugs (days 45 to 47) the animals were put into a round aluminum container measuring 50 cm in diameter and 30 cm height, and a door bell on top of the container was sounded for 1 min. The convulsive reactions of the animals to the sound were graded 0 to 4 according to Schreiber and Schlensinger (1972).

In a second phase, these animals continued to be treated up to day 73. Marihuana was now injected i.p. twice daily, 20 mg/kg each injection; the control solution was also injected i.p. twice daily (1.0 ml/kg). Barbitone sodium continued to be administered through the drinking bottles. From days 74—76 the drugs were withheld and audiogenic sensitivity was again assessed as in phase one.

In both phases body weight was recorded on the last day before withholding the drug and after 1—3 days of abstinence.

Results

Experiment 3. Twenty four to 96 h of abstinence from previous daily administration of 80 mg/kg of marihuana extract for 36 days (about 27 mg/kg Δ^9-THC daily) did not induce hypersensitivity to cardiazol in the rats (Table 4). On the contrary, they were significantly more resistant to convulsion. Thus, after 24, 48 and 96 h of abstinence it took significantly larger doses of cardiazol and more time elapsed before the appearance of the first convulsion. Furthermore, 4 of the cannabis-

Table 4. Convulsions and death induced by cardiazol in abstinent rats from previous i.p. treatment with marihuana extract or control solutions for 36 days

Chronic treatment	Days of abstinence	First convulsion		Number of survivals after 125 mg/kg cardiazol/number of animals tested
		Time of appearance (min)	Dose of cardiazol (mg/kg)	
0.9% NaCl	1	7.5 ± 2.4	57.5 ± 12.0	0/10
Tween-80	4	9.2 ± 2.8	55.0 ± 15.8	0/10
Marihuana	1	13.6 ± 4.2*	88.8 ± 22.0*	1/9
Marihuana	2	16.5 ± 3.6*	86.3 ± 18.1*	2/9
Marihuana	3	9.2 ± 2.7	58.4 ± 12.5	0/9
Marihuana	4	16.7 ± 10.7*	77.8 ± 19.5*	1/9

* Differs significantly from respective control group (P at least ≤ 0.05; Student t-test).

Table 5. Audiogenic convulsions of rats abstinent from previous marihuana extract and barbitone sodium administration

Phase	Drug previously administered	Mean dosage during 7 last days (mg/kg/daily)	Days of abstinence	Total number of convulsions/ number of animals tested	Number of animals showing convulsions graded as		
					1	2	3
1	Control sol.	—	1	1/7	1	0	0
	Control sol.	—	2	0/6	0	0	0
	Barbitone Na	314	1	4/10	0	0	4
	Marihuana	29	1	2/9	1	0	1
	Marihuana	28	2	0/9	0	0	0
	Marihuana	32	3	0/8	0	0	0
2	Control sol.	—	1	1/6	1	0	0
	Control sol.	—	3	0/7	0	0	0
	Barbitone Na	342	2	7/10	0	0	7
	Marihuana	40	1	2/10	1	0	1
	Marihuana	40	2	0/7	0	0	0
	Marihuana	40	3	0/9	0	0	0

treated animals survived to the maximum dose of cardiazol against none of the controls (Table 4). As a side observation it was noted that 3 of the rats receiving cannabis developed a sizable enlargement of their abdomen after the first week of treatment which, however, disappeared with the continuation of the treatment.

Experiment 4. Table 5 shows that one control animal showed audiogenic convulsions during 1 to 3 days of abstinence from previous chronic administration of control solution for 44 (phase 1) and 73 (phase 2) days. In rats with similar periods of previous administration and withdrawal of marihuana sound challenge induced convulsions in only 2 animals. These results contrast with the 4 and 7 animals which convulsed from the barbiturate group. On the other hand, control and marihuana groups did not lose weight after 1—3 days of abstinence.

Discussion

Forced self administration of cannabis extracts for 4 months did not lead the animals to a "cannabis directed behavior" (Experiment 1), as has been described for opiates (Nichols, 1963; Stolerman and Kumar, 1970), even when they were 1 to 4 days in abstinence (Fig. 1). The same failure was obtained when the animals were first trained to drink the marihuana suspension in order to be rewarded with sweetened milk (Experiment 2). These data suggest that marihuana does not act in rats

either as a primary reinforcer (to alleviate eventual abstinence symptoms) or as a secondary reinforcer through pairing it with food. In this respect cannabis contrasts with other drugs which are self administered by rats such as morphine, codeine, cocaine, methamphetamine, nicotine, pipradrol, amobarbital, hexobarbital, chlordiazepoxide and ethyl alcohol (Schuster and Thompson, 1969). However, two other interpretations are also possible. First, Δ^9-THC is eliminated rather slowly by rats (Klausner and Dingell, 1971) and the 96 h withdrawal used could not be enough to induce abstinence symptoms and to originate self-administration. Second, Δ^9-THC has been shown to induce the "bait shyness" phenomenon (Elsmore and Fletcher, 1972); the aversion to marihuana resulting from it could be greater than the aversive symptoms of the eventual withdrawal syndrome, thus blocking self-administration.

The long term administration of marihuana, 4 months by the oral route (Experiment 1), or 36 days through the i.p. route (Experiment 3) did not induce signs of toxicity. Only a decrease of body weight was noted at the end of treatment. In the case of oral ingestion (Experiment 1) this was due to the continuous decrease of liquid intake as the animals rapidly regained weight after 5 days of access to the control solution (Table 3).

The transitory enlargement of the abdomen observed in a few rats under chronic i.p. treatment with the rather large dose of 80 mg/kg daily could be due to the described irritation of the peritoneum induced by some cannabis preparations (Manning, McDonough, Elsmore, Salles, and Sodetz, 1971).

Our results also indicate that there is no abstinence syndrome after withdrawal of marihuana in chronically treated rats. None of the symptoms present in abstinence from morphine, such as, piloerection, "wet dog shakes", increased defecation, irritability and aggressiveness, were observed in the marihuana abstinent rats (Experiment 1). The symptoms of barbiturate abstinence syndrome, convulsions and dramatic fall in body weight, were also absent (Table 5). On the other hand, the marihuana-abstinent rats were more resistant to cardiazol than controls. This could be due to an increased amount of liver metabolizing enzymes induced by cannabis (Witschi and Saint-François, 1972; Sofia and Barry, 1973) which rapidly inactivated cardiazol, decreasing its toxicity. Another possibility is that cardiazol was less well absorbed from the peritoneal cavity due to the irritation caused by cannabis. Finally, we did not observe in our rats any particular sign or behavior which could be considered as a peculiar symptom of marihuana abstinence syndrome. The absence of a withdrawal syndrome in pigeons and rats receiving large doses of Δ^9-THC for about 1 month has recently been described (McMillan, Harris, Frankenheim, and Kennedy, 1970; Harris, 1971).

Our results are in contrast with those reported by Kaymakçalan (1972) showing physical dependence and abstinence syndrome to Δ^9-THC. Physical dependence and abstinence was shown in Rhesus monkeys after a 1.6 mg/kg daily dose of Δ^9-THC for 3—8 weeks. Our rats received a dose approximately 10 times larger by the oral route during 4 months (Experiment 1) or 17 times greater by the i.p. route during 36 days. Therefore, we believe that neither the dosage nor the duration of the chronic administration could explain the discrepancy. As the species used and the methodology employed were so different, no further comparison between the experiments is possible.

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