Pharmacology Biochemistry & Behavior, Vol. 5, pp. 269–273.

Role of Experience in Acquisition and Loss of Tolerance to the Effect of Δ-9-THC on Spaced Responding[1]

FREDERICK J. MANNING

Walter Reed Army Institute of Research, Washington, D.C. 20012

(Received 9 March 1976)

MANNING, F. J. *Role of experience in acquisition and loss of tolerance to the effects of Δ-9-THC on spaced responding.* PHARMAC. BIOCHEM. BEHAV. 5(3) 269–273, 1976. — Albino rats were given extensive training in spaced responding, using a DRL 30 sec schedule of food reinforcement (only lever presses more than 30 sec apart were reinforced). All rats then went 12 days without behavioral testing. During this period half the rats received daily intragastric doses of delta-9-tetrahydrocannabinol (THC) and the rest equal volumes of the THC vehicle. On day 13, some rats received THC 3 hr before behavioral testing while others received only vehicle. The former showed a sharp increase in lever press rate over baseline levels, but the vehicle control rats were unaffected. The rats with 12 prior THC doses were no less affected than those with no previous drug history. Continued testing resulted in recovery of baseline performance within 5 sessions, again with no effect of previous drug history. Similar results were obtained with doses of 4 mg/kg and 16 mg/kg, though the drug's effects were more pronounced at the higher dose. These results demonstrate that performance in the drug state can be a far more important determinant of tolerance than mere exposure to THC. Drug administration was then suspended for 1 week. Rats that had become tolerant to 4 mg/kg THC were then redivided into 3 new groups. One group received daily doses of vehicle and DRL sessions, a second received DRL sessions without vehicle, and 1 group received neither vehicle nor DRL sessions for this week. Subsequent DRL testing after THC administration showed that only the groups receiving DRL sessions in the intervening week lost their previously acquired tolerance. Experience thus appears to play an important role in loss of tolerance to THC as well as in acquisition of tolerance.

Tetrahydrocannabinol Tolerance DRL schedule

ONE OF the most striking features of research on the behavioral effects of marihuana and its constituents has been the attenuation of many quite substantial changes in behavior with chronic administration. McMillian and his associates, for example, have shown tolerance to behavioral effects of Δ-9-tetrahydrocannabinol (THC) in pigeons and rats even greater than that which develops with repeated doses of morphine [14].

Not all behavioral effects of THC or marihuana show such tolerance however (e.g., [3,12]), and several recent experiments are very difficult to reconcile with traditional explanations of drug tolerance such as changes in drug distribution, metabolism, and excretion. Carder and Olson [5] provided a particularly important instance in their examination of tolerance to the depressant effects of a marihuana extract on the lever pressing of rats for water reinforcement. Rats receiving marihuana extract before each behavioral testing session pressed significantly less on the first day of drug treatment, but showed complete tolerance by the sixth such test. Rats receiving marihuana over the same period, but after each session rather than before, showed little or no evidence of behavioral tolerance when the drug was subsequently administered before testing. They concluded that mere application of marihuana, without providing the experience of responding while drugged, was insufficient to develop behavioral tolerance. The generality of this finding might be questioned however, since the authors could not produce parallel findings using food reinforcement, and others [10] have since reported quite different results in similar work using a shock avoidance task. The present work extends this line of inquiry from the relatively non-specific sedation noted in the above experiments to the stimulating effect of THC on lever pressing controlled by a DRL (differential-reinforcement-of-low-rates) schedule of reinforcement. This effect has been reported in a variety of species, at doses at least as low as any in the literature [8, 11, 12, 18]. It is also one of the few which has been reported for human [4] as well as

[1] In conducting the research described in this report, the investigators adhered to the Guide for the Care and Use of Laboratory Animals DHEW Publication No. (NIH) 73-23, as prepared by the Institute of Laboratory Animal resources, National Research Council.

non-human subjects, and is consistent with frequent human reports of altered temporal perception after marihuana smoking.

METHOD

Animals

Forty male albino rats of the Wistar-derived barrier reared Walter Reed strain were used. All were allowed to grow 380 g, then reduced to 300 g and kept at this weight through the experiment. All were individually caged and had water available at all times in these cages. Food earned during behavioral testing was supplemented by varying amounts placed in the home cage immediately after testing.

Apparatus

Four identical BRS-Foringer operant chambers were used for this experiment. Equal numbers of experimental and control rats were assigned to each chamber, and each rat was tested in the same chamber throughout the experiment. Each chamber had a single lever on one wall, to the right of a centrally located food hopper, into which 45 mg Noyes food pellets were dispensed. A single small pilot light 5 cm over the lever and two 5 W house lights at the top of the opposite wall were illuminated throughout the session, except while the lever was depressed.

Drugs

Synthetic Δ-9-THC was made available by the National Institute of Drug Abuse. Supplied in ethanol at a concentration of 0.2 g/ml, it was diluted with propylene glycol in amounts sufficient to allow dosing with a constant volume of 0.3 ml. Vehicle doses consisted of 0.3 ml of a propylene glycol-ethanol solution in which the volume of ethanol was equal to the volume of the stock THC solution in the corresponding THC dose. Route of administration was intragastric (via feeding tube) and took place 3 hr prior to the scheduled start of each rat's daily testing session.

Procedure

When the body weight of each rat reached 300 g, it was given its first behavioral testing session. Each lever press response was reinforced, for a total of 50, or until 50 min had elapsed. Any rats not obtaining 50 pellets in this session were given further such sessions on succeeding days until responding was established. The reinforcement schedule was then changed to DRL 10 sec, DRL 20 sec, and finally DRL 30 sec over a course of 10–20 sessions. Twenty-five sessions were run under the DRL 30 sec schedule, the last 10 of which were preceded by 3 hr by doses of THC vehicle. The top half of Table 1 outlines the design of the experiment from this point on.

At this point behavioral testing was suspended for 12 days. During this period half the subjects (Groups 4-4, 16-V, and 16-16) received daily oral doses of Δ-9-THC, and half (Groups V-V, V-4, and V-16) continued to receive daily oral doses of the vehicle. Of those receiving THC, 12 rats (Group 4-4) received 4 mg/kg and 8 (Groups 16-V and 16-16) received 16 mg/kg. Dose volume was always 0.3 ml. When behavioral testing was again resumed, Groups V-4, 4-4, V-16 and 16-16 received THC 3 hr prior to testing, V-4 and 4-4 receiving 4 mg/kg and V-16 and 16-16 receiving 16 mg/kg. Groups 16-V and V-V received only the vehicle. Dosing and testing continued for 5 days.

At the conclusion of 5 days of DRL testing under the influence of THC, Phase 1 ended and Phase 2 began. The bottom half of Table 1 outlines the procedures for this phase. Briefly stated, the vehicle controls and the 16 mg/kg rats were dropped from the experiment at this point, and all clearly tolerant rats from Groups V-4 and 4-4 were maintained drug free for the following 7 days. One-third of these rats (chosen at random) were left undisturbed in their home cages for this period. This is Group R, for rest, in Table 1. Another third (selected similarly) received doses of vehicle and DRL sessions each day. This is Group SV, for sessions and vehicle, in Table 1. The remaining rats (Group S) were given DRL sessions without vehicle administration. On the 8th day of this phase, all rats were given a DRL session 3 hr after administration of Δ-9-THC, 4 mg/kg.

TABLE 1

OUTLINE OF SEQUENCE OF FINAL TRAINING AND DRUG CONDITIONS

		Procedure		
Phase I		10 sessions	12 days rest	5 sessions
Group	n	of DRL 30		of DRL 30
V-4	12	vehicle	vehicle daily	4 mg/kg THC
4-4	12	vehicle	THC 4 mg/kg daily	4 mg/kg THC
V-16	4	vehicle	vehicle daily	16 mg/kg THC
16-16	4	vehicle	THC 16 mg/kg daily	16 mg/kg THC
16-V	4	vehicle	THC 16 mg/kg daily	vehicle
V-V	4	vehicle	vehicle daily	vehicle
Phase II				
Group	n	7 days		1 session of DRL 30
R	5	rest, no vehicle		4 mg/kg THC
S	5	DRL 30, no vehicle		4 mg/kg THC
SV	5	DRL 30, vehicle daily		4 mg/kg THC

RESULTS

Phase 1

Table 2 summarizes the performances of the various groups during the final 3 baseline sessions (vehicle 3 hr prior) and the 5 THC sessions. Group means are presented for 3 measures: total responses in the sessions, the median interresponse time, and the ratio of reinforcements to responses (absolute no. of reinforcements was not very informative, since most sessions by definition consisted of 50 reinforcements). Baseline values on all three variables are similar for all groups, an observation confirmed by one-way analyses of variance. Collapsing across the pretreatment variable, the data from the first drug session of all the rats receiving vehicle on this day (V-V and 16-V) were compared to those receiving 4 mg/kg (V-4 and 4-4) or 16 mg/kg (V-16 and 16-16). T-tests confirmed the drug effects obvious by inspection of Table 1: rats receiving 16 mg/kg were significantly ($p<0.01$) more affected by THC than those receiving 4 mg/kg, who in turn differed significantly ($p<0.01$) from those receiving only vehicle. Since the primary interest of the experiment lay in possible differences between groups pretreated with THC (16-V, 16-16, 4-4) and those receiving only vehicle (V-V, V-16,V-4), separate 2-way analyses were conducted com-

TABLE 2

GROUP MEANS (± S.E.M.) OF SEVERAL PERFORMANCE MEASURES DURING FINAL 3 BASELINE AND 5 DRUG SESSIONS*

Groups	Mean Baseline	Consecutive THC Sessions 1	2	3	4	5
Total Responses						
V-V	118 ± 16	117 ± 15	118 ± 12	84 ± 19	87 ± 18	86 ± 19
16-V	107 ± 14	106 ± 13	107 ± 15	73 ± 21	75 ± 20	79 ± 22
16-16	121 ± 17	192 ± 8	155 ± 17	117 ± 24	123 ± 23	126 ± 20
V-16	114 ± 17	147 ± 20	124 ± 22	115 ± 15	112 ± 21	89 ± 21
4-4	120 ± 14	145 ± 9	125 ± 13	128 ± 16	116 ± 13	117 ± 16
V-4	123 ± 15	159 ± 16	157 ± 17	135 ± 16	123 ± 14	129 ± 16
Median Interresponse Time						
V-V	31.2 ± 1.8	30.8 ± 2.0	30.9 ± 1.9	33.6 ± 3.6	32.9 ± 2.9	33.3 ± 2.7
16-V	30.9 ± 2.0	32.0 ± 2.9	30.6 ± 2.7	34.7 ± 3.3	34.9 ± 3.7	34.9 ± 3.3
15-16	29.0 ± 1.9	20.0 ± 1.8	24.2 ± 2.4	29.5 ± 2.9	28.0 ± 3.6	28.2 ± 3.5
V-16	29.2 ± 1.9	22.0 ± 3.2	27.7 ± 1.3	28.4 ± 1.6	26.5 ± 3.0	33.8 ± 4.9
4-4	29.5 ± 1.9	25.8 ± 1.3	28.2 ± 1.5	29.4 ± 2.3	29.4 ± 2.3	30.6 ± 2.3
V-4	27.8 ± 2.4	22.7 ± 2.1	23.4 ± 1.9	25.4 ± 2.1	27.1 ± 2.3	27.3 ± 2.3
Reinforcement/Response						
V-V	0.45 ± 0.08	0.47 ± 0.08	0.48 ± 0.09	0.61 ± 0.11	0.59 ± 0.12	0.58 ± 0.13
16-V	0.50 ± 0.12	0.52 ± 0.12	0.52 ± 0.10	0.65 ± 0.13	0.65 ± 0.15	0.62 ± 0.14
16-16	0.42 ± 0.09	0.18 ± 0.02	0.25 ± 0.08	0.46 ± 0.08	0.41 ± 0.05	0.47 ± 0.09
V-16	0.42 ± 0.09	0.29 ± 0.09	0.39 ± 0.11	0.39 ± 0.06	0.40 ± 0.11	0.55 ± 0.15
4-4	0.42 ± 0.07	0.29 ± 0.05	0.35 ± 0.06	0.42 ± 0.08	0.43 ± 0.07	0.46 ± 0.09
V-4	0.43 ± 0.08	0.26 ± 0.04	0.30 ± 0.08	0.34 ± 0.09	0.40 ± 0.04	0.38 ± 0.09

*Groups V-V and 16-V received only the THC vehicle during the "THC Sessions"

paring baseline vs 1st day drug session performances of pretreated and non-pretreated groups at each dose level, i.e., 16-V vs V-V, 16-16 vs V-16, and 4-4 vs V-4. Results were similar on each of the 3 dependent variables: a highly significant drug effect ($F(1,6)$ or $(1,22) \geq 21.80$; $p<0.01$ in all cases) for all groups receiving THC 3 hr prior to the 1st drug session, but no pretreatment effect or interaction (all $F's \leq 1.39$; df = 1,6 or 1,22; n.s.). The 12 day suspension of training did not significantly affect performance, since neither of the groups receiving only vehicle 3 hr prior to drug session 1 differed from baseline on any measure ($F(1,6) = 1.18$; n.s.). Similar analyses were applied to the data from the 5 drug sessions, to detect any possible effects of pretreatment on tolerance development. Again, however, on all 3 variables, there was no significant effect of pretreatment ($F's \leq 1.82$; df = 1,6 or 1,22; n.s.). There was a strong effect of sessions for all groups receiving THC 3 hr prior to the sessions (all $F's \geq 5.20$; df = 4,24 or 4,88; $p<0.01$ in all cases), indicating tolerance development, but no interaction with pretreatment ($F's \leq 1.27$; df = 4,24; or 4,88).

Phase 2

Since Groups V-4 and 4-4 did not differ in any way in Phase 1, these rats were reassigned after the 5th session under THC, into 3 groups: R (rest), S (sessions), and SV (sessions after vehicle). Since this phase was meant to investigate loss of tolerance, only rats showing both a clear cut drug effect (>10% deviation from baseline median IRT) and unequivocal tolerance (fifth THC session median IRT >90% baseline) were included. To recapitulate the procedure, Group R then rested in home cages for 7 days, while Groups S and SV were run in daily DRL sessions. Rats in Group SV received a vehicle gavage, 3 hr prior to these sessions. On day 8 all rats received a DRL session 3 hr after a 4 mg/kg dose of THC.

Table 3 shows performance measures for the rats in their fifth THC session, after tolerance had developed, and their sixth THC session, 1 week later. It is clear that only the rats of Group R, who did not perform the DRL task during this week, were still fully tolerant in the sixth session. Both of the other groups, who had undergone behavioral testing during the intervening week, showed significant drug effects during the sixth THC session. Groups S and SV did not differ from each other, so vehicle administration probably did not contribute to loss of tolerance to any extent.

DISCUSSION

In agreement with earlier findings by Bueno and Carlini [3], Carder and Olson [5], and Manning [11,12], the present experiments show that mere exposure to THC, in this case at a dose as large as 16 mg/kg for 12 days, in the absence of behavioral testing while drugged, is not sufficient to produce tolerance to a behavioral effect of THC (i.e. reduce the size of the effect relative to that in drug naive rats). The fact that both groups regained baseline performance levels within 5 days when performing the DRL task while in the drug state suggests that the 12 day pretreatment was not an unreasonably short pretreatment period. The fact that the performances of Groups V-V and 16-V were unaffected by 12 days' without testing is evidence that the poor performances of all the other groups

TABLE 3

PERFORMANCES OF TOLERANT RATS BEFORE AND AFTER A ONE WEEK DRUG-FREE PERIOD

	Fifth THC Session			Sixth THC Session		
Rat #	Med IRT	Resp.	Rein/Res	Med IRT	Resp.	Rein/Resp
1	22.7	173	0.24	20.3	188	0.22
3	32.9	93	0.54	20.4	171	0.20
4	36.0	82	0.61	29.8	110	0.46
6	46.8	58	0.86	39.8	56	0.89
7	37.3	78	0.64	28.9	110	0.45
$\overline{X}_S \pm$ S.E.M.	35.1 ± 4.4	97 ± 22	0.58 ± 0.11	27.8 ± 4.0*	127 ± 26†	0.44 ± 0.14†
9	21.1	184	0.07	18.8	218	0.06
10	33.6	74	0.68	32.7	81	0.62
13	34.0	76	0.66	32.9	79	0.63
14	34.8	71	0.70	25.6	100	0.50
15	33.1	81	0.62	27.6	154	0.27
$\overline{X}_{SV} \pm$ S.E.M.	31.3 ± 2.9	97 ± 24	0.55 ± 0.14	27.5 ± 2.9†	126 ± 30†	0.42 ± 0.12‡
17	35.9	65	0.77	40.6	66	0.76
20	22.8	127	0.13	23.7	132	0.20
21	34.3	70	0.72	25.1	119	0.34
22	23.8	136	0.32	23.3	131	0.33
23	33.5	71	0.70	33.9	80	0.64
$\overline{X}_R \pm$ S.E.M.	30.1 ± 3.1	94 ± 17	0.52 ± 0.26	29.3 ± 3.8	106 ± 15	0.48 ± 0.23

*Differs from fifth THC session with $p<0.01$ (t-test for paired obs.)
†Differs from fifth THC session with $p<0.05$ (t-test for paired obs.)
‡Differs from fifth THC session with $p<0.01$ (t-test for paired obs.)

in THC Session 1 was due to THC and not merely the result of 12 days without testing.

While the present results by no means rule out the operation of purely pharmacological mechanisms of tolerance, they do make it clear that performance in the drug state is a far more rapid means of developing tolerance to the effect of THC on spaced responding than is mere exposure to THC. Ferraro [8], Sodetz [18], and Manning [11,12] have previously pointed out the advantages of viewing at least certain instances of tolerance as learned adjustments to a THC-caused increase in error rate. This view also provides a plausible explanation of why some THC effects do not seem to show tolerance – they are not detrimental to the subject. The present results are certainly compatible with this assessment. They might also be described in terms of state-dependent learning [15], though it is not clear that this explanation differs materially from the learned adjustment view. The Phase II results of the present experiment also favor one or both of these theories over more traditional mechanisms, since they indicate that just as tolerance development may depend more upon practicing in the drug state than upon drug exposure per se, loss of tolerance may be more affected by what the animal does during abstinence than by mere abstinence itself.

Although the present findings, and the learned adjustment viewpoint in general, might be compatible with a homeostatic and redundancy theory of tolerance like that proposed by Martin [13], it is clear that the present findings add to a growing list of experiments suggesting that the traditional explanations of tolerance in terms of drug distribution and metabolism or receptor sensitivity are not entirely adequate in the case of THC. Studies with amphetamine [16] alcohol [6] mescaline [2], chlordiazepoxide [7], and morphine [1, 9, 17] suggest that this may be true with many other drugs as well. It seems likely that only an integration of pharmacological data with an assessment of the environmental variables supporting behavior will permit complete specification of a drug's behavioral effects.

ACKNOWLEDGEMENTS

The author is grateful to Mason C. Jackson, Jr., Josef Curiel, and Cathy Simmons for skillful technical assistance, to Wilhelmina Taylor for preparation of the manuscript, and to Frank J. Sodetz for much helpful discussion.

REFERENCES

1. Adams, W. J., S. Y. Yeh, L. A. Woods and C. L. Mitchell. Drug-test interaction as a factor in the development of tolerance to the analgesic effect of morphine. *J. Pharmac. exp. Ther.* **168**: 251–257, 1969.
2. Bridger, W. H., I. J. Mandel and D. M. Stoff. Mescaline: no tolerance to excitatory effects. *Biol. Psychiat.* 7: 129–138, 1973.
3. Bueno, O. F. A. and E. A. Carlini. Dissociation of learning in marihuana tolerant rats. *Psychopharmacologia* **25**: 49–56, 1972.
4. Cappell, H., C. D. Webster, B. S. Herring and R. Ginsberg. Alcohol and marihuana: A comparison of effects on a temporally controlled operant in humans. *J. Pharmac. exp. Ther.* **182**: 195–203, 1972.
5. Carder, B. and J. Olson. Learned behavioral tolerance to marihuana in rats. *Pharmac. Biochem. Behav.* **1**: 73–76, 1973.
6. Chen, C. S.: A study of the alcohol-tolerance effect and introduction of a new behavioral technique. *Psychopharmacologia* **12**: 433–440, 1968.

7. Christensen, J. D. Tolerance development with chlordiazepoxide in relation to the plasma levels of the parent compound and its main metabolites in mice. *Acta pharmac. tox.* **33**: 262–272, 1973.
8. Ferraro, D. Effects of Δ-9-tetrahydrocannabinol on simple and complex learned behavior in animals. In: *Current Research in Marihuana,* edited by M. L. Lewis, New York: Academic Press, 1972, pp. 49–50.
9. Heifetz, S. A. and D. E. McMillan. Development of behavioral tolerance to morphine and methadone using the schedule-controlled behavior of the pigeon. *Psychopharmacologia* **19**: 40–52, 1971.
10. Johansson, J. O., B. G. Henriksson and T. U. C. Jarbe. Effects of Δ-8-THC on dissociation of conditioned avoidance responding in tolerant and nontolerant rats. *Physiol. Psychol.* **2**: 431–432, 1974.
11. Manning, F. J. Acute tolerance to the effects of delta-9-tetrahydrocannabinol on spaced responding by monkeys. *Pharmac. Biochem. Behav.* **1**: 665–671, 1973.
12. Manning, F. J. Chronic delta-9-tetrahydrocannabinol: transient and lasting effects on avoidance behavior. *Pharmac. Biochem. Behav.* **4**: 17–21, 1976.
13. Martin, W. R. A homeostatic and redundancy theory of tolerance to and dependence on narcotic analgesics. In: *The Addictive States,* edited by A. Wilker, Baltimore: The Williams & Wilkins Co., 1968, pp. 206–225.
14. McMillan, D. E., W. L. Dewey and L. S. Harris. Characteristics of tetrahydrocannabinol tolerance. *Ann. N. Y. Acad. Sci.* **191**: 83–99, 1972.
15. Overton, D. A. Dissociated learning in drug states (state-dependent learning). In: *Psychopharmacology: A Review of Progress 1957–1967,* edited by D. H. Efron *et al.*, USPHS Publication No. 1836, Washington: U.S. Government Printing Office, 1968, pp. 918–930.
16. Schuster, C. R., W. S. Dockens and J. H. Woods. Behavioral variables affecting the development of amphetamine tolerance. *Psychopharmacologia* **9**: 170–182, 1966.
17. Siegal, S. Evidence from rats that morphine tolerance is a learned response. *J. comp. physiol. Psychol.* **89**: 498–506, 1975.
18. Sodetz, F. J. Δ-9-tetrahydrocannabinol: behavioral toxicity in laboratory animals. In: *Current Research in Marihuana,* edited by M. L. Lewis. New York: Academic Press, 1972, pp. 25–48.