Pharmacology Biochemistry and Behavior, Vol. 1, pp. 665–671. ANKHO International Inc., 1973. Printed in the U.S.A.

Acute Tolerance to the Effects of Delta-9-Tetrahydrocannabinol on Spaced Responding by Monkeys[1]

FREDERICK J. MANNING[2]

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

(Received 23 April 1973)

MANNING, F. J. *Acute tolerance to the effects of delta-9-tetrahydrocannabinol on spaced responding by monkeys.* PHARMAC. BIOCHEM. BEHAV. 1(6) 665–671, 1973. -- Rhesus monkeys were trained to lever press for food reinforcement on a differential-reinforcement-of-low-rates (DRL) schedule, then given six different doses, *per os*, of Δ-9-tetrahydrocannabinol (THC), ranging from 0.5–16 mg (0.07–2.86 mg/kg). Relative to vehicle placebos, all six doses produced increases in both the number of unreinforced responses and the time required to obtain 60 reinforcements and decreases in the median interresponse time. In addition, marked pausing occurred after the higher doses. In 20 of 24 drug sessions these performance changes were less prominent in the second half of the session. In a second experiment the nature of this within-session improvement was investigated by comparing performances beginning 3 hr after THC ingestion, as in Experiment 1, with those beginning 4 hr afterwards. In all cases performance resembled those of Experiment 1, suggesting that it is performance under the influence of THC rather than mere exposure to the drug that is responsible for the marked improvement in performance observed during drug sessions.

Tetrahydrocannabinol DRL schedule Rhesus Tolerance

RESEARCH on the behavioral effects of marihuana has increased explosively in the few years since the isolation and synthesis of a major active constituent, Δ-9-tetrahydrocannabinol (THC). This outpouring has banished some myths about marihuana, but has also produced almost as many new questions about the drug as it has answers. The area of tolerance is one notable example. Human marihuana devotees have often been less affected than inexperienced users on experimental tasks [11, 14, 19] but they do not seem to require increasingly larger doses to achieve desired subjective effects [11,19]. In fact, chronic users typically appear more sensitive to these effects than do inexperienced users. Non-human subjects have shown a similar inconsistency in this regard. Although increased sensitivity, or reverse tolerance has not yet been successfully demonstrated, repeated administrations of THC to non-human subjects has produced dramatic attenuation of drug effects on, for example, operant key-pecking by pigeons [13] or lever pressing by rats [1] or chimpanzees [7]. On the other hand, tolerance to some effects has been conspicuously absent [e.g., 10], even in animals showing pronounced tolerance to one or more other effects [1, 8, 15].

The experiments reported below are the first of a series aimed at specifying (a) the circumstances under which tolerance may be expected to develop, and (b) the mechanism underlying such tolerance as does develop.

EXPERIMENT 1

Three different laboratories [5, 6, 9] have independently reported that the operant performance of chimpanzees maintained under differential-reinforcement-of-low-rates schedules (DRL schedules) was disrupted by doses of THC in the effective human dose range, which is far lower than the range typically used with non-human subjects [8, 10, 13]. Although all three laboratories interpreted their findings as suggesting that chimpanzees are exceptionally well-suited for research with THC, similar findings by Pradhan, Bailey, and Ghosh [17] with rats suggest that it is the DRL schedule instead which is so well-suited for THC research. The experiment reported here, using rhesus

[1] In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Science – National Research Council.

[2] The author thanks Mason C. Jackson, Jr. for his assistance in all phases of this research, and Wilhelmina Taylor for her help in preparation of the manuscript.

monkeys, seems to favor this view, and at the same time provides an example of very rapid tolerance to Δ-9-THC. The nature of this tolerance is studied further in Experiment Two.

Method

Animals. Four male rhesus monkeys *(Macaca Mulatta)* were used. Three were older juveniles, weighing approximately 6 8 kg, and one (K681), slightly younger, weighed 5.6 kg. The older animals had previously participated in a brief experiment utilizing a DRL schedule, but the young monkey was experimentally naive. In addition, one of the older juveniles (L28) had previously suffered extensive ablation of orbitofrontal cortex. His data are included here only because they are so similar to those of the other three animals.

Apparatus. Animals were individually housed in wire cages enclosed in sound attenuating experimental chambers (BRS-Foringer) constructed from 3.8 cm marine grade harborite sealed with epoxy resin. Cage dimensions were approximately 35 x 48 x 52 cm. Doors to these chambers were closed only during daily testing sessions. During these sessions a portable intelligence panel was mounted on the front of the animal's cage. This panel, contructed of fiberboard with a stainless steel veneer, measured 43 x 43 cm. Mounted 8 cm from the top, equidistant from the sides, was a small pilot light with jeweled reflector. Directly below this, a toggle switch extended 3.8 cm into the monkey's cage. Centered at the very bottom of the panel was a circular hole, 8 cm in dia, through which the monkey could reach a small reinforcement cup. At appropriate occasions, 750 mg Noyes monkey food pellets were delivered to this cup. Programming and data recording were accomplished from an adjacent room with solid state logic modules, electromechanical counters, and a cumulative recorder.

Procedure. All monkeys were tested five days per week. Each session lasted until the subject had obtained 60 food pellets, or a maximum of 3 hr. After initial shaping a DRL schedule was in effect for the balance of the experiment. The length of the minimum reinforced interresponse time was slowly increased to 60 sec. Data collected were session length, total responses and interresponse times (IRT's). The last of these were automatically grouped in 12-sec class intervals (bins) for two animals and 6-sec class intervals for the other two. When an animal showed stable performance on this procedure (i.e., no change in modal IRT bin for 10 sessions), drug administration began.

Synthetic Δ-9-THC, supplied in ethanol by the National Institute of Mental Health, was further diluted with ethanol to yield concentrations such that the volume administered was always 0.2 cc. Placebos consisted of 0.2 cc of ethanol. Both THC doses and placebos were administered by injecting the liquid into an 8 cm piece of banana and handing it to the monkey to eat 3 hr before his session began. The animals were always observed closely until they had at least put the fruit into their mouths. Inspection of feces pans under each cage never revealed any evidence that the banana was rejected after observation had ceased. Six different doses of Δ-9-THC were given to the monkeys, in a different random order for each animal. Absolute dosing was employed (i.e., each animal received the same amount of THC, regardless of body weight), and the doses formed a geometric series from 0.5 mg to 16.0 mg. Table 1 shows these doses, converted to relative dosage (mg/kg) for easier comparison to previous work, along with the orders of administration. At least a week elapsed between successive doses, and all non-drug sessions were placebo sessions.

TABLE 1

RELATIVE DOSES (mg/kg) AND THEIR ORDER OF ADMINISTRATION FOR EACH ANIMAL

	THC in mg/kg (order of receipt)			
THC in mg	H363	K681	H090	L28
0.5	0.07 (5)	0.09 (4)	0.08 (3)	0.06 (3)
1.0	0.15 (4)	0.18 (2)	0.17 (5)	0.13 (6)
2.0	0.29 (3)	0.36 (6)	0.33 (6)	0.26 (2)
4.0	0.59 (1)	0.71 (1)	0.67 (2)	0.52 (5)
8.0	1.18 (2)	1.43 (5)	1.33 (4)	1.04 (1)
16.0	2.35 (6)	2.86 (3)	2.67 (1)	2.08 (4)

Results

Three summary measures of performance, session length, median IRT, and errors (IRT's<60 sec), were converted to difference measures by subtracting from the values obtained during the drug session the values obtained in the immediately preceding placebo session. Table 2 displays the difference measures obtained for each animal. With one exception every drug administration in this study resulted in an increase in errors and a decrease in median IRT. The one exception was L 28, the heaviest animal in the study, at the smallest dose, 0.5 mg. In relative terms, this monkey received 0.07 mg/kg of THC, which is reputed to be the minimally effective oral dose for humans [18]. The only reliable dose-effect relationship involved session length. There was general tendency for the drug sessions to be longer (i.e., the monkeys took longer to obtain 60 pellets) as dosage increased. This apparent relationship is misleading, however, since longer sessions may result from three quite different changes in behavior. First, the subject may shift his entire response distribution toward longer IRT's. Second, he may shift his distribution toward shorter IRT's and thereby increase his number of errors, each of which may add up to 60 sec to his session time. Finally, he may emit a few very long pauses or simply stop responding altogether. The subjects in this experiment extended their low dose session lengths only by making more errors, and extended their high dose session lengths by a combination of more errors and one or more very long pauses (greater than 5 min). The latter were seen only at the 2 highest dose levels employed. Thus the continuous increase in session length as a function of dose is not produced by a single continuous dose-dependent process, but by the overlapping of two qualitatively different drug effects.

Figure 1 displays IRT distributions for all four animals after three selected doses of THC (1, 4, and 16 mg), along with the preceding placebo sessions. Omitted, for brevity and clarity only, are data from sessions following 0.5, 2, and 8 mg. For two animals available instrumentation al-

TABLE 2

DIFFERENCE SCORES (DRUG SESSIONS–PLACEBO SESSIONS) FOR EACH OF THREE PERFORMANCE MEASURES AT EACH OF SIX DOSES OF THC FOR EACH OF FOUR RHESUS MONKEYS

	Drug Session - Control Session											
	Errors				Median IRT (sec)				Session Length (sec)			
THC (mg)	H363	K681	H090	L28	H363	K681	H090	L28	H363	K681	H090	L28
0.5	34	20	37	−6	−4.2	-12.8	−6.6	1.0	1465	3195	-827	−351
1.0	14	18	7	15	−4.9	- 4.2	−6.6	−1.5	499	462	- 91	730
2.0	12	3	15	31	−2.2	- 3.5	−4.3	3.9	709	186	550	1634
4.0	69	26	20	35	−5.6	−10.7	-3.0	-5.1	3420	1537	746	1345
8.0	57	*	39	23	−4.7	-- 0.4*	−5.9	−2.2	2946	4334*	1324	5940
16.0	147	31	29	30	-8.5	-22.9	−5.0	−3.7	7311	3151	1026	5940

*K681 emitted only 10 responses

lowed collection of IRT's in 6-sec bins, but in two others 12-sec bins were used. A leftward shift toward shorter IRT's is characteristic of THC sessions in all four sets of graphs. This shift appears most clearly in the 2 cases with 6-sec IRT bins, but this is probably a measurement artifact due to the magnitude of the shift itself being much closer to 6 sec than 12 sec. That the shapes of the distributions were not drastically changed by THC (i.e., they are still unimodal, bell-shaped, and centered approximately around the minimum reinforced IRT) suggests that the DRL contingency was still exerting powerful control over the animal's behavior. The drug effect was primarily a systematic error in the direction of shorter IRT's. Considerable inter- and intra-animal variability (even more pronounced when all 6 doses for each animal are considered) again precludes any generalization relating magnitude of drug effect to dose of THC.

Figure 2, which presents representative cumulative records, reveals an additional characteristic of the effects of THC on spaced responding: the distribution of errors is not at all random. Instead, a marked within-session improvement is obvious. In 20 of 24 sessions the majority of the errors were committed before the subject gained half his 60 reinforcements. Averaging over all subjects and all drug sessions, 68% of the total errors committed occurred in the first half of the session and only 32% in the second half.

Discussion

In general, the results obtained conform quite well to those reported in studies employing rat [17] and chimpanzee [5, 6, 9] subjects. This agreement emerges despite a substantial number of procedural differences among these studies. It is, in fact, difficult to specify common elements in these 5 experiments besides the use of the DRL contingency. Further testimony to the sensitivity of spaced responding to THC lies in the very low dosages effective in the present experiment. In three of the four subjects, effects were produced by doses of less than 0.10 mg/kg. This is very close to the minimum effective oral dose in humans [18], and ten to one hundred times smaller than the dosage effective in typical experiments with non-human animals [8, 10, 13]. In this respect, the present results are clearly at variance with the suggestions of some [6,9] that the chimpanzee offers some unique advantage, in terms of sensitivity, for the study of Δ-9-THC. The present results are also in agreement with the findings of Cappell *et al.* [2], who used a DRL schedule to assess the effects of Δ-9-THC in humans. They are also consistent with anecdotal and experimental reports [4,12] of "altered temporal perception" by human marihuana users (i.e., they report "60 seconds has passed" after only 50 sec). The spaced responding generated by DRL schedules thus appears to provide an excellent baseline for assessing the presence and nature of tolerance to Δ-9-THC: the drug produces similar effects in a variety of species, including man, and these are seen at doses as small or smaller than any heretofore reported.

The major new observation reported in this experiment is that the increase in unreinforced responses produced by THC is largely confined to the first half of the session (approx. 1 hr). The drugged animal quite often had reattained his baseline proficiency by the end of a single 60-reinforcement testing session. Experiment 2 addressed the nature of this rapid recovery.

EXPERIMENT 2

The work described below was a rather simple test of two of the most obvious possible explanations of the highly skewed error distributions seen in Experiment One. It is possible that the relative scarcity of errors beyond the first hour of testing (3rd to 4th hour postingestion) closely reflected the time course of the drug's action - the resultant of the interaction of absorption, metabolism, and excretion. On the other hand, this within-session improvement may have resulted from the interaction of the drugged animal with his external environment, and may be viewed

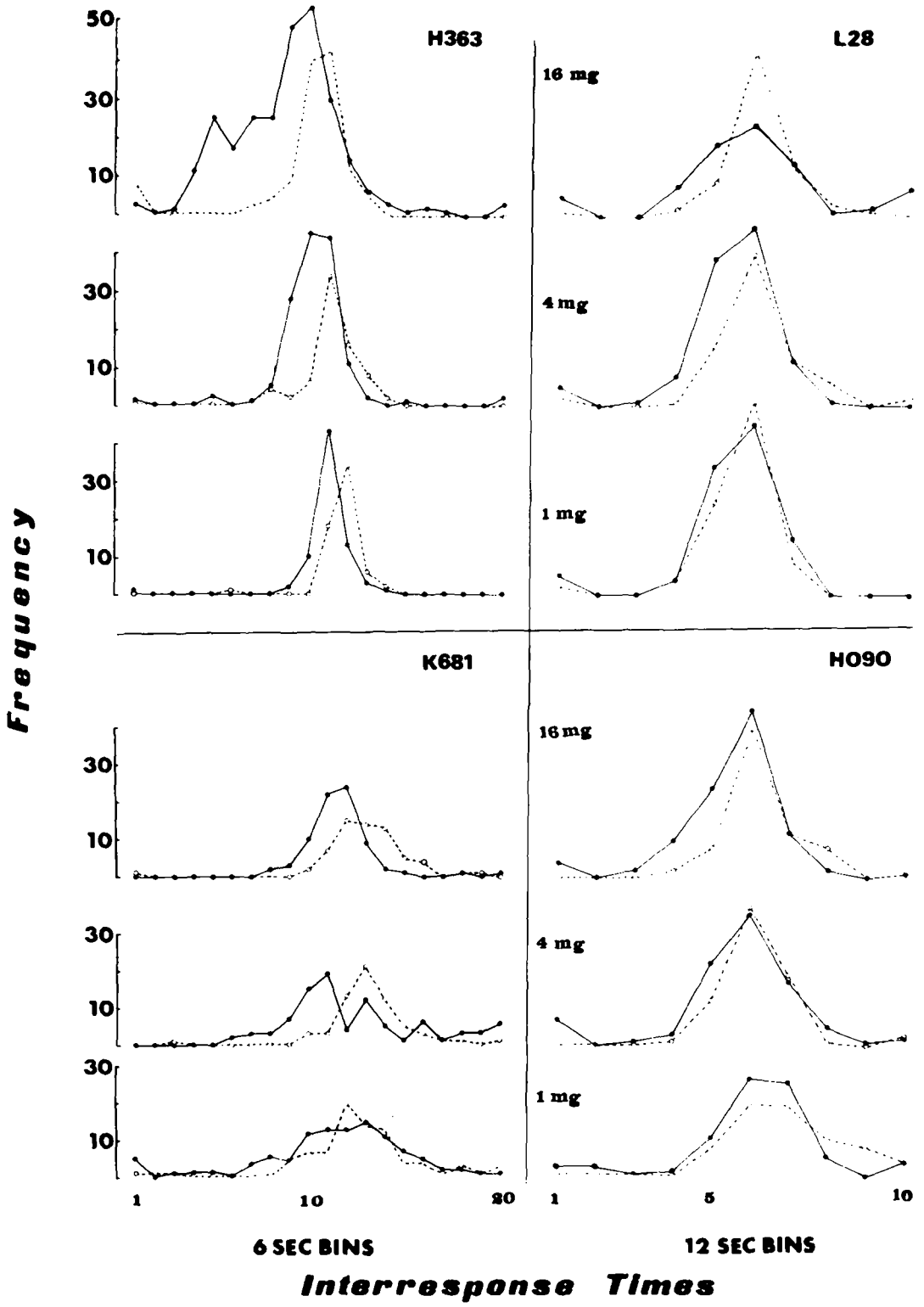

FIG. 1: Interresponse time distributions for each of the four animals (H363, H090, K681, and L28) on 3 selected THC days (solid lines) and their corresponding placebo days (dashed lines). Drug sessions displayed followed 1, 4, and 16 mg.

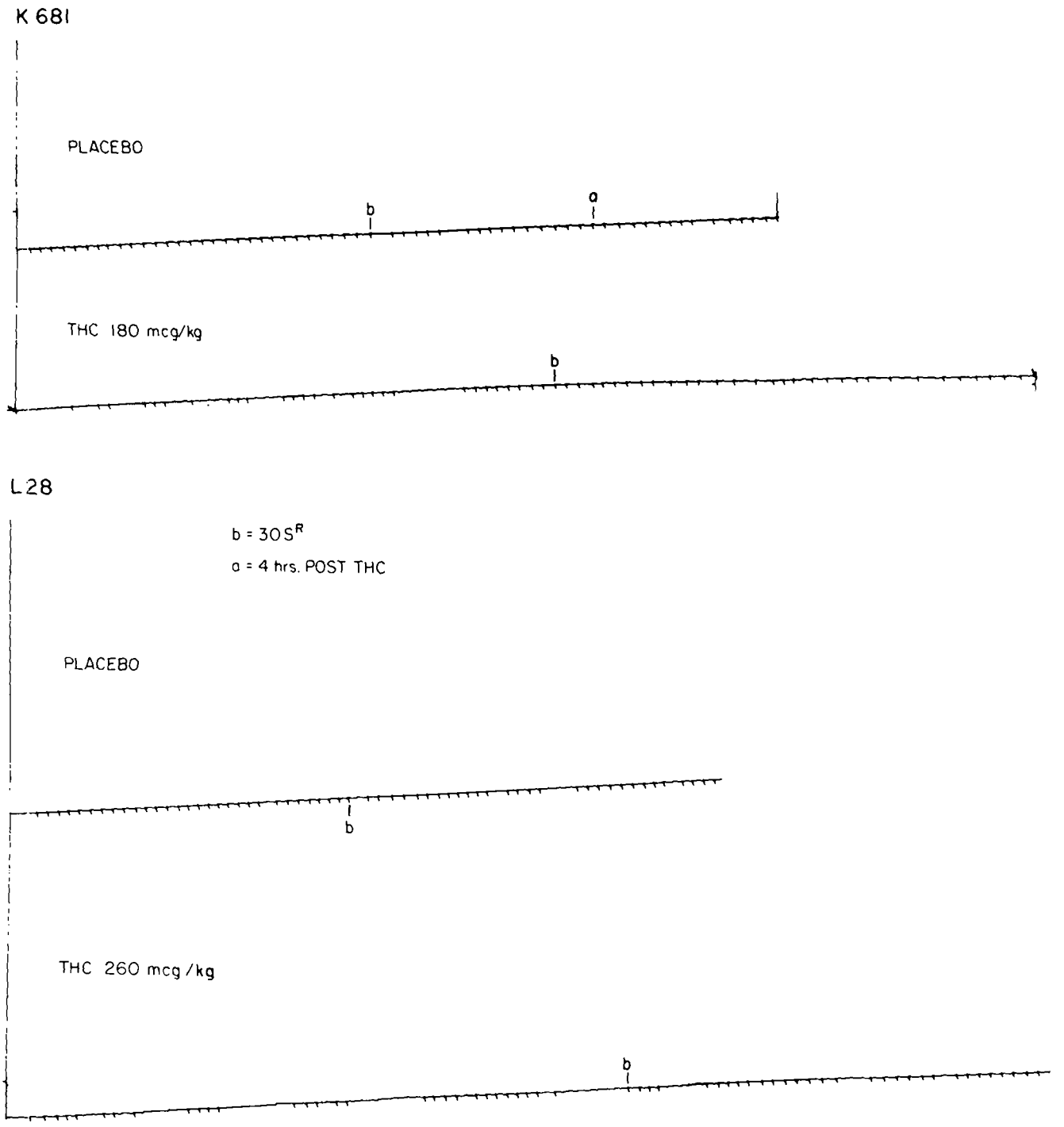

FIG. 2: Representative cumulative records, from two animals (K681 and L28), of performance under the DRL 60-sec schedule after placebo and after Δ-9-THC.

as the adaptive response of a food-deprived organism to a suddenly decreased frequency of reinforcement.

These two positions are of course not mutually exclusive, but some measure of the relative importance of exposure to THC *per se* and exposure to the reinforcement contingencies as well may be gained by holding the former constant while varying the latter.

Method

Animals. Three of the four monkeys from Experiment 1

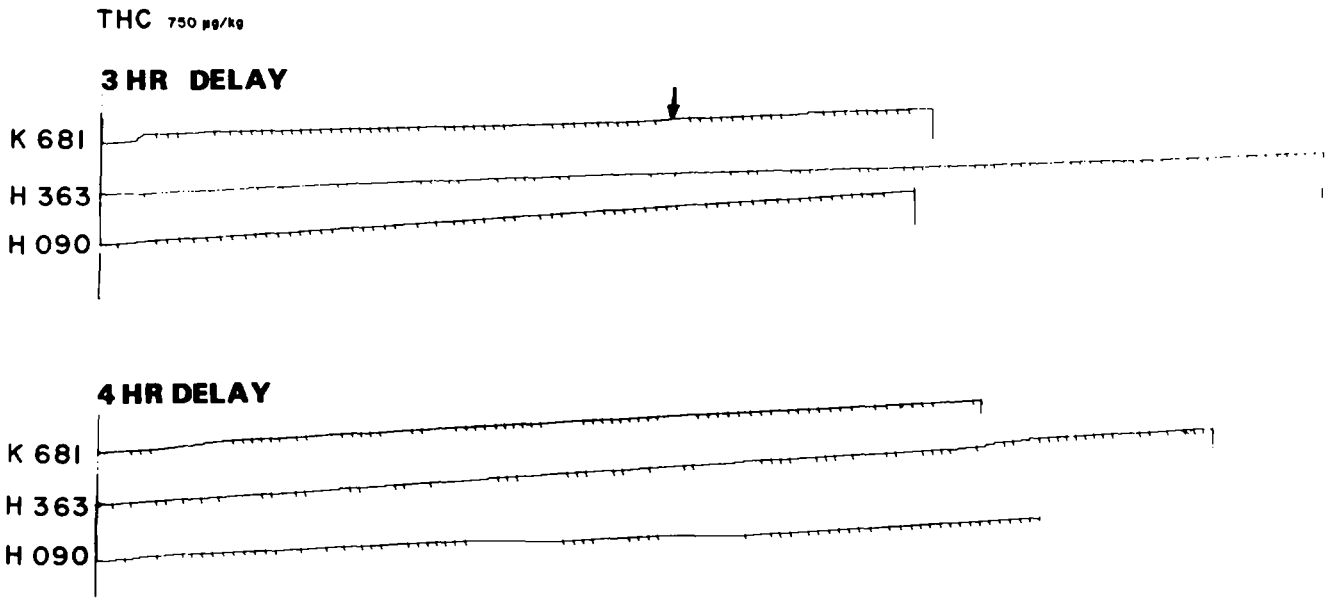

FIG. 3: Cumulative records of the performances under the DRL 60-sec schedule of all three animals after 0.75 mg/kg THC, 3 and 4 hr prior to the beginning of the session. Arrow indicates one hr into session.

were used. Monkey L28 was not used in this experiment. Monkey H090 had not received THC for a period of 6 months, and K681 and H363 had not received the drug for 3 weeks and 2 weeks respectively.

Apparatus and Procedures. These were identical to those of Experiment 1 in all respects except the following. Animals received only two treatment with Δ-9-THC, both of which were doses of 0.75 mg/kg, PO. On one of these two occasions, THC ingestion preceded the start of the behavioral testing by 3 hr, as in Experiment 1. The start of the other drug session followed ingestion by 4 hr. If the peak of drug action occurs 3 hr or less after ingestion, sessions starting at 4 hr after ingestion should be far less disrupted by THC than those starting 3 hr after THC. For two animals (K681 and H363) and the 3-hr delay condition occurred before the 4 hr delay. For the other animal (H090) conditions were reversed.

Results

Figure 3 displays the cumulative records of all the relevant sessions (performances after the placebos were highly similar to those displayed in Fig. 2). It is clear from the similarity of the 2 sets of records that delaying the onset of behavioral testing until 4 hr after THC ingestion is considerably less effective as a method of eliminating errors than is allowing the monkey to interact with the contingencies of reinforcement for that extra hour. In fact, analysis of Table 3, which presents efficiency measures (reinforcements per response) for each monkey during and after the first hour of performance, suggests that an extra hour's exposure to THC fails to diminish its disruptive effects on spaced responding at all: efficiency during the first hour of the sessions starting 4 hr after THC ingestion is no better than during the first hour of sessions beginning 3 hr after ingestion. On the other hand, the efficiency of every monkey was considerably higher after one hour's performance, regardless of the time since THC ingestion.

TABLE 3

EFFICIENCY OF PERFORMANCE UNDER A DRL SCHEDULE 3 AND 4 HR AFTER INGESTION OF Δ^9-THC (0.75 mg/kg)

		Reinforcements Per Response			
		3 Hr Delay		4 Hr Delay	
S		First Hr	After 1 Hr	First Hr	After 1 Hr
K681	drug	0.61	0.79	0.51	0.72
	placebo	0.94	0.91	0.98	0.94
H363	drug	0.27	0.58	0.38	0.55
	placebo	0.88	0.73	0.79	0.78
H090	drug	0.75	0.83	0.65	0.87
	placebo	0.98	0.93	0.92	0.93

Discussion

This experiment provides a clear demonstration that the rapid recovery from the disruptive effects of Δ-9-THC on spaced responding by rhesus monkeys is not a result of mere exposure to the drug, but is critically dependent upon the interaction of the subject with the reinforcement contingencies. Under these circumstances the most parsimonious explanation of the observed tolerance seems to be the law of effect: the drugged monkey is controlled by the same tendency to optimize reinforcement frequency as is any other food-deprived organism. Decreases in reinforcement density lead to compensatory adjustments in behavior. In this view the rapid within-session improvement

displayed by drugged subjects is a learning curve, much like the rapid improvement shown by non-drugged animals adapting to an increase in the length of the minimum reinforced interresponse time. (We have, in fact, generated cumulative records very similar to those in Fig. 3 merely by changing from a DRL 60-sec schedule to a DRL 72-sec schedule).

It is difficult to specify with confidence the relationship between this rapid within-session tolerance and the slower across-session tolerance observed by numerous other investigators. It is undeniable that some pharmacological tolerance to Δ-9-THC must occur, at least in the pigeon, simply because it is difficult to imagine how learning could underlie the upward shift in the lethal dose produced by THC pretreatment [13]. However, it is also undeniable that more than a few behavioral experiments are very difficult, if not impossible, to explain on this basis alone. Carder and Olson [3] for example have shown that rats administered THC daily demonstrated tolerance to its suppressant effects on bar pressing only if they were allowed to press while drugged. Pirch and his colleagues [16] have shown that THC enhanced the shuttle-box avoidance performance of some rats (those with poor baseline performances) and decreased the performance of others (those with excellent baseline performances). Only the rats in which acute administration of THC resulted in decreased avoidance showed any evidence of tolerance when injections were continued. Finally, Ferraro [7] has reported that 2 mg/kg Δ-9-THC produced large decreases in the operant response rates of monkeys working for food under a variable-interval schedule. Only when these rate decreases produced a significant decrease in reinforcement frequency was tolerance observed, and the lowered response rates which did increase with repeated injections did so only until the baseline reinforcement frequency was reattained. Explanations of tolerance to THC which emphasize absorption, distribution, metabolism, sensitivity of target tissue, or excretion all handle these data, as well as those of the present experiment, only with extreme difficulty. However, they are entirely consistent with the learning hypothesis expressed here, that a substantial proportion of tolerance to behavioral effects of THC is due to the general tendency of organisms to maximize reinforcement density.

REFERENCES

1. Bueno, O. F. A. and E. A. Carlini. Dissociation of learning in marihuana tolerant rats. *Psychopharmacologia (Berl.)* **25**: 49–56, 1972.
2. 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.
3. Carder, B. and J. Olson. Learned behavioral tolerance to marihuana in rats. *Pharmac. Biochem. Behav.* **1**: 73–76, 1973.
4. Clark, L. D., R. Hughs and E. N. Nakashima. Behavioral effects of marihuana. *Archs gen. Psychiat.* **23**: 193–198, 1970.
5. Cole, J. M., W. A. Pieper and D. M. Rumbaugh. Effects of Δ-9-tetrahydrocannabinol on spaced responding in great apes. *Communs. Behav. Biol.* **6**: 285–293, 1971.
6. Conrad, D. G., T. F. Elsmore, and F. J. Sodetz. Δ-9-tetrahydrocannabinol: dose-related effects on timing behavior in chimpanzee. *Science* **175**: 547–550, 1972.
7. Ferraro, D. P. Effects of Δ-9-Trans-tetrahydrocannabinol on simple and complex learned behavior in animals. In: *Current Research in Marihuana*, edited by M. L. Lewis, New York: Academic Press, 1973.
8. Ferraro, D. P. and D. M. Grilly. Lack of tolerance to Δ-9-tetrahydrocannabinol in chimpanzees. *Science* **179**: 490–492, 1973.
9. Ferraro, D. P., Grilly and W. C. Lynch. Effects of marihuana extract on the operant behavior of chimpanzees. *Psychopharmacologia (Berl.)* **22**: 333–351, 1971.
10. Ham, M. Ten, and J. van Noordwijk. Lack of tolerance to the effect of two tetrahydrocannabinols on aggressiveness. *Psychopharmacologia (Berl.)* **29**: 171–176, 1973.
11. Jones, R. T. Tetrahydrocannabinol and the marhuana-induced social "high", or the effects of the mind on marihuana. *Ann. N. Y. Acad. Sci.* **191**: 155–165, 1971.
12. Jones, R. T. and G. C. Stone. Psychological studies of marihuana and alcohol in man. *Psychopharmacologia (Berl.)* **18**: 108–117, 1970.
13. McMillan, D. E., W. L. Dewey and L. S. Harris. Characteristics of tetrahydrocannabinol tolerance. *Ann. N. Y. Acad. Sci.* **191**: 83–96, 1971.
14. Meyer, R. E., R. C. Pillard, L. M. Shapiro and S. M. Mirin. Administration of marijuana to heavy and casual marijuana users. *Am. J. Psychiat.* **128**: 90–96, 1971.
15. Pirch, F. H., R. A. Cohn, P. R. Barnes and E. S. Barratt. Tolerance to EEG and behavior effects of marihuana in rats. In: *Drug Addiction: Volume 1 Experimental Pharmacology*, edited by J. M. Singh, L. Miller and H. Lal, Mount Kisco, New York: Futura, 1972.
16. Pirch, J. H., K. C. Osterholm, E. S. Barratt and R. A. Cohn. Marihuana enhancement of a shuttle-box avoidance performance in rats. *Proc. Soc. exp. Biol. Med.* **141**: 590–592, 1972.
17. Pradhan, S. N., P. T. Bailey and P. Ghosh. Effects of Δ-9-tetrahydrocannabinol on a timing behavior in rats. *Psychon. Sci.* **27**: 179–181, 1972.
18. United States Department of Health, Education, and Welfare. *Marihuana and Health: Second Annual Report to Congress.* Washington, D. C.: U. S. Government Printing Office, 1972.
19. Weil, A. T., N. E. Zinberg and J. M. Nelson. Clinical and psychological effects of marhuana in man. *Science* **162**: 1234–1242, 1968.