Effects of delta⁸-tetrahydrocannabinol (THC) on avoidance extinction in rats

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Using a fixed behavioral test—resistance to extinction of avoidance responding in rats in the Baum box—the effects of delta⁸-THC were examined with the drug at various degrees of decomposition. Partially decomposed THC had no significant overall effect on avoidance-extinction responding. However, substantially decomposed THC produced significant extreme reactions in one group of rats, with some subjects showing virtually no extinction responding, whereas others displayed greatly increased responding. In Experiment 2, pure delta⁸-THC was found to significantly hasten extinction, but only if rats had never experienced the drug before (i.e., a tolerance effect was demonstrated). Drug dosage throughout was 5 mg/kg.

Baum (1965) developed an automated ledge box for the avoidance training of rats. In the box, the rats' avoidance responses were characterized by rapid acquisition and a fair amount of resistance to extinction. The resistance to extinction of the response in this apparatus has proven useful in studies of various pharmacological agents, when a drug is administered following acquisition and prior to extinction. Baum (1969) found that ethanol administered in this way greatly prolonged responding in extinction. Similar "paradoxical" effects were found with chlordiazepoxide and diazepam (Ziskind, Amit, & Baum, 1974). Nicotine effects, however, were nonsignificant (Tremblay & Baum, 1985).

Using hashish resin and the same paradigm, Jaffe and Baum (1971) found that the drug had a very marked effect toward increasing extinction responding. The present study sought to further explore the effects of cannabis on avoidance extinction in rats. Since hashish and cannabis vary greatly in chemical composition (Grlic, 1964), this study employed an isomer of tetrahydrocannabinol (THC), namely delta⁸-THC, one of the major psychoactive constituents of cannabis (Mechoulam, 1970). The dose of

delta⁸-THC was held constant throughout this study at 5 mg/kg. Using an equivalent dose of delta⁸-THC, no effect on acquisition of conditioned fear was found (Gonzalez, Karniol, & de Carlini, 1972). In general, the results of THC's on avoidance performance have been conflicting and contradictory (McMillan, 1977).

The effects of hashish resin on avoidance extinction in rats referred to above (Jaffe & Baum, 1971) are rather surprising, given the high dosage employed. These findings may be due to changes in drug composition resulting from lengthy storage or due to the fact that those rats had never experienced hashish before. The present study was designed to see what effect drug changes in delta⁸-THC due to storage, or, alternatively, familiarity with prior THC injections had on the effects of delta⁸-THC on avoidance extinction in rats.

EXPERIMENT 1

This experiment explored whether delta⁸-THC that was deliberately exposed to heat and light would have different behavioral effects than THC which had been carefully stored.

Method

Subjects. The subjects were 33 experimentally naive female hooded rats obtained from the Canadian Breeding Farm, St. Constant, Quebec. They were maintained with free access to food and water in their home cages and, at the time of testing, weighed 150–200 g and seemed in good health.

Apparatus. The apparatus used was an automated one-way avoidance box or Baum box, very similar to the original prototype (Baum, 1965). It was the same piece of equipment used in other studies of drugs carried out in this laboratory (Baum, 1969; Tremblay & Baum, 1985), and is redisplayed graphically in a recent paper (Baum, 1986). Shock was

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delivered to the grid floor of the box from a commercially made scrambled shock source (Grason-Stadler), and shock intensity was always 0.5 mA.

Procedure. Avoidance acquisition was identical to that in previous studies (Tremblay & Baum, 1985), with a CS-UCS interval of 10 sec (interval available for avoiding) and an intertrial interval of 30 sec, spent on the safety ledge. There was no prior habituation to the avoidance apparatus, and no discrete warning signal was employed in avoidance training.

The drug consisted of 500 mg of (-)-trans-delta⁸-THC (99%) dissolved in ethanol and sealed in an ampoule under nitrogen. It was supplied by the Department of National Health and Welfare (Canada) and was refrigerated and protected from light until its arrival in our laboratory. Upon arrival, the ampoule was broken and its contents were added to a 250-cc solution of 95% propylene glycol (by volume) and 5% ethanol. This was the same vehicle used previously for hashish resin in an earlier, similar study (Jaffe & Baum, 1971). Of the final prepared solution, 124 cc were placed in an opaque plastic bottle and refrigerated, for use with Group THC rats; the remainder was placed in a transparent plastic bottle and placed in a plastic basket with a thick cardboard lid. A 150-W electric light bulb was placed 15 cm above this remaining solution, and the temperature in the basket was maintained at 55°–60° C. This part of the solution was thus exposed to heat and light for use with Group THC-DEC. The exposure to heat and light, which was deliberately designed to produce some decomposition of the THC, lasted 90 consecutive hours, after which this solution was refrigerated.

After attaining the avoidance-acquisition criterion of 10 consecutive avoidance responses in the Baum box, each rat was assigned to one of three groups, each having $n=11$, and removed from the avoidance box. Group THC rats received a 5-mg/kg injection of THC solution, Group THC-DEC rats received an injection of 5 mg/kg of the "decomposed" THC solution, and Group PBO received a placebo injection of the drug vehicle only. All injections were intraperitoneal. Following injection, the rat was placed in a plastic pail for a 20-min waiting period.

After the waiting period following injection, each rat was returned to the avoidance box for extinction testing; the shocker was disconnected so shock could no longer be delivered to the grid floor. Extinction testing began with an intertrial interval (i.e., with the rat's being placed on the safety ledge), and proceeded until the rat attained an extinction criterion of 5 consecutive minutes on the grid without responding, or until an upper limit of 200 responses was made.

Results

Three nonparametric tests were used to analyze the results: The Mann-Whitney U test, the Kruskal-Wallis one-way analysis of variance by ranks, and the Moses Test of Extreme Reactions (Siegel, 1956). Acquisition was similar for all three groups, and there were no significant differences on the number of shocks received or number of acquisition trials required to reach the learning criterion (Kruskal-Wallis $H_s < 0.60$, $p > .10$). Overall, acquisition was completed in a mean of 16.3 trials, 4.0 of which were escapes (shocks received).

The mean number of extinction responses made by the three groups were as follows: Group PBO, 39.7 (range 0–114); Group THC, 51.0 (range 5–200); and Group THC-DEC, 63.2 (range 0–189). Although there was a tendency for the drug to prolong extinction responding, the differences were not significant ($H=0.02$, $p > .10$; U tests of two groups at a time, $U_s > 58$, $ps > .05$). However, although Group THC-DEC did not make significantly more responses in extinction, in reality it consisted of some rats which made very few or no responses and others which made a great many. Thus, a Moses Test of Extreme Reactions was significant when comparing

Group THC-DEC and Group PBO ($p=.0005$) and approached significance in the comparison of Groups THC and THC-DEC ($p=.09$).

Chemical analysis of the drug solutions. The two separate solutions of (-)-trans-delta⁸-THC, one having been deliberately exposed to heat and light, were subjected to qualitative and quantitative chemical analysis, with the help of Krystof Jankowski of the Department of Chemistry, Université de Moncton. Qualitative analysis was done with thin-layer chromatography, the results of which are shown in Table 1. If the drug had been pure, two stains would have appeared on the chromatograms, corresponding to THC and glycol. However, as seen in Table 1, there were four substances instead of two in solution THC and six substances in solution THC-DEC. This indicates that solution THC had undergone some decomposition in the course of the study, whereas solution THC-DEC (deliberately exposed to heat and light) had undergone even more.

The identification of the components and their concentrations were obtained by spectroscopic analysis and are shown in the lower portion of Table 1. It is also possible that there were traces of other substances. The decomposition of solution THC-DEC is understandable and was deliberately induced. Contamination of solution THC was inadvertent.

Discussion

The results, using delta⁸-THC rather than the hashish resin previously employed (Jaffe & Baum, 1971), show no reliable effect of THC to either increase or decrease responding in extinction in the Baum box. However, both THC solutions were contaminated, one intentionally so. The THC deliberately decomposed by heat and light resulted in extreme reactions in extinction responding within the group of rats given THC-DEC. An analogous effect on avoidance extinction in a highly similar paradigm was found previously with diazepam doses of 1 mg/kg and 3 mg/kg (Ziskind et al., 1974). As in the present study, the extreme reactions were found when rats were injected with the drug in question for the first time. No extreme reactions were found in rats previously habituated to diazepam injections (Ziskind et al., 1974).

Table 1
Summary of Thin-Layer Chromatography and Spectroscopic Analysis of Solutions THC and THC-DEC of Experiment 1

THC	THC-DEC
Chromatography	
11.4 (strong)	11.3 (strong)
	43.6 (weak)
	54.7 (weak)
66.0 (weak)	64.5 (weak)
73.6 (medium)	74.2 (medium)
81.5 (medium)	82.2 (medium)
Spectroscopic Analysis	
28% delta ⁸ -THC	25% delta ⁸ -THC
16% delta ⁸ -THC 11 - hydroxy	12% delta ⁸ -THC 11 - hydroxy
16% delta ⁸ -THC 8,11 - dihydroxy	9% delta ⁸ -THC 7alpha,11 - dihydroxy
40% glycol	40% glycol
	8% delta ⁸ -THC 7beta,11 - dihydroxy (probably)
	6% product which is not THC

EXPERIMENT 2

Experiment 2 was designed to test the effect of totally uncontaminated delta⁹-THC on avoidance extinction in rats in the Baum box. In addition, since the effect of drugs on avoidance extinction may be dependent on the novelty of being drugged for the first time, for example with ethanol (Amit, Ziskind, & Baum, 1973) and diazepam (Ziskind et al., 1974), prior exposure to delta⁹-THC was also investigated. There is some controversy over whether animals become tolerant (Newman, Lutz, Gould, & Domino, 1972) or sensitized (Smith, Davis, & Pace, 1972) when THC is administered at regular intervals. There is also the question of whether the rats must perform the behavioral task while drugged or whether mere drug administration is enough to produce tolerance (Manning, 1976). The usual conclusion is that tolerance is easily obtained with few exposures to THC (McMillan, 1977). Experiment 2 was designed to verify this, in addition to testing the effects of pure uncontaminated THC on extinction of avoidance responding.

Method

Subjects and Apparatus. The subjects were an additional 41 experimentally naive female hooded rats. The apparatus was the same as in Experiment 1, with shock intensity again being 0.5 mA.

Procedure. An additional ampoule of 500 mg of delta⁹-THC was obtained from the Department of National Health and Welfare (Canada). The THC solution was prepared as for solution THC in Experiment 1, and was placed in an opaque bottle, hermetically sealed, and kept refrigerated. Chemical analysis carried out both before and after the experiment was conducted revealed the drug to be 99% pure and uncontaminated.

The procedure was similar to that of Experiment 1, except that four groups of rats were run in a 2×2 factorial design. One factor was pure THC versus placebo injected prior to extinction. The other factor was preexposure to THC versus preexposure to placebo injections. Preexposure consisted of three injections of THC (always 5 mg/kg) or placebo on 3 consecutive days at 24-h intervals prior to avoidance acquisition. In all other respects, acquisition and extinction procedures were identical to those of Experiment 1, except that the upper limit on extinction responding was arbitrarily chosen as 300 responses (vs. 200 in Experiment 1). The four groups were Groups THC-P (drugged prior to extinction, preexposed to THC, $n=11$); THC-N (drugged prior to extinction, no preexposure to THC, $n=9$); PBO-P (placebo, preexposed to placebo injections, $n=11$) and PBO-N (placebo, no prior injections, $n=10$).

Results and Discussion

Because extinction scores were not normally distributed, nonparametric statistics were used in the analysis of the results. Once again, there were no acquisition differences among the four groups (Kruskal-Wallis $H_s < 2.73$, $ps > .20$, for the same acquisition measures as in Experiment 1). The number of responses made in extinction was quite variable; the means and ranges were as follows: Group PBO-P, 41.9 (0-156); Group PBO-N, 56.4 (0-132); Group THC-P, 56.4 (0-300); and Group THC-N, 6.2 (0-29). In comparing two groups at a time with Mann-Whitney U tests on the number of extinction responses, there was a significant difference between Groups THC-N and PBO-N, with Group THC-N making significantly fewer responses ($U=19$, $p < .05$).

Other comparisons were not significant. Thus, pure uncontaminated THC acts to hasten avoidance extinction and diminish responding. Furthermore, there is some evidence for a tolerance effect. The effect that some tolerance is obtained after only three prior injections of THC is consistent with other findings (McMillan, 1977). Since THC-P animals were drugged outside the behavioral test situation, these findings contradict to some extent those of Manning (1976).

In conclusion, the results of this study indicate that the effects of delta⁹-THC (5 mg/kg) on avoidance extinction depend on the degree of purity of the drug. If the THC is pure, it hastens avoidance extinction. If it is partially decomposed, it has no effect; and if decomposition is extensive, extreme reactions result. Furthermore, tolerance is readily obtained. These results are different from those found with hashish resin in a similar paradigm (Jaffe & Baum, 1971), demonstrating indeed that the behavioral pharmacology of the THC's is a difficult one (McMillan, 1977, p. 29).

REFERENCES

- AMIT, Z., ZISKIND, D., & BAUM, M. (1973). Drug effects and avoidance extinction in rats: A test of the drug novelty hypothesis using ethanol. *Animal Learning & Behavior*, 1, 41-43.
- BAUM, M. (1965). An automated apparatus for the avoidance training of rats. *Psychological Reports*, 16, 1205-1211.
- BAUM, M. (1969). Paradoxical effect of alcohol on the resistance-to-extinction of an avoidance response in rats. *Journal of Comparative Psychology*, 69, 238-240.
- BAUM, M. (1986). An animal model for agoraphobia using a safety-signal analysis. *Behavior Research & Therapy*, 24, 87-89.
- GONZALEZ, S. C., KARNIOL, T. G., & DE CARLINI, E. L. (1972). Effects of *Cannabis sativa* extract on conditioned fear. *Behavioral Biology*, 7, 83-94.
- GRILIC, L. (1964). Recent advances in the chemical research of cannabis. *Bulletin of Narcotics*, 16, 29-38.
- JAFFE, P. G., & BAUM, M. (1971). Increased resistance-to-extinction of an avoidance response in rats following the administration of hashish resin. *Psychopharmacologia*, 20, 97-102.
- MANNING, F. J. (1976). Role of experience in acquisition and loss of tolerance to the effect of delta⁹-THC on spaced responding. *Pharmacology, Biochemistry, & Behavior*, 5, 269-273.
- McMILLAN, D. E. (1977). Behavioral pharmacology of the tetrahydrocannabinols. In T. Thompson & P. B. Dews (Eds.), *Advances in behavioral pharmacology* (Vol. 1, pp. 1-34). New York: Academic Press.
- MECHOULAM, R. (1970). Marihuana chemistry. *Science*, 168, 1159-1166.
- NEWMAN, M. L., LUTZ, M. P., GOULD, M. H., & DOMINO, E. F. (1972). Delta⁹-tetrahydrocannabinol and ethyl alcohol: Evidence for cross-tolerance in the rat. *Science*, 175, 1022-1023.
- SIEGEL, S. (1956). *Nonparametric statistics for the behavioral sciences*. Toronto: McGraw-Hill.
- SMITH, S. G., DAVIS, W. M., & PACE, H. (1972). Delta⁹-tetrahydrocannabinol in pigeons: Increasing sensitivity to its effects on variable-interval performance. Paper read at Southeastern Psychological Association Meeting, Atlanta, GA.
- TREMBLAY, M., & BAUM, M. (1985). Effects of nicotine on the resistance to extinction of a jump-up avoidance response in rats. *Bulletin of the Psychonomic Society*, 23, 341-342.
- ZISKIND, D., AMIT, Z., & BAUM, M. (1974). Resistance to extinction of an avoidance response in rats following the administration of chlor-diazepoxide (Librium) or diazepam (Valium). *Psychopharmacologia*, 38, 231-238.