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Behavioral Effects in Monkeys of Racemates of Two Biologically Active Marijuana Constituents

Abstract. *Both* dl-Δ^8- *and* dl-Δ^9-*tetrahydrocannabinol produced marked alterations of behavior in rhesus and squirrel monkeys. Squirrel monkeys appeared to have visual hallucinations. Continuous avoidance behavior of squirrel monkeys was stimulated by both drugs, but high doses of* dl-Δ^9-*tetrahydrocannabinol also caused depression after the stimulant phase. Complex behavior involving memory and visual discrimination in rhesus monkeys was markedly disrupted by both drugs.*

Pharmacologic studies of *Cannabis sativa* (marijuana, hashish) in animals have produced inconsistent results (*1–3*). Hashish induced aggressiveness (fighting) in one study (*2*) but prevented aggressive behavior in another (*4*). Some work has indicated that hashish produced analgesia, but this effect was not always found (*3, 4*). Apart from these diverse findings the main variable requiring control is the purity or composition of the hashish (*5, 6*). The actual amounts of the tetrahydrocannabinols (THC) contained in the hashish administered to animals have been difficult to determine because THC decomposes when exposed to air (*7*). A standard tetrahydrocannabinol preparation is needed to aid in the evaluation of the action of marijuana or hashish. Fahrenholtz, Lurie, and Kierstead (*8*) have synthesized crystalline *dl*-Δ^9-tetrahydrocannabinol, the racemate of the major active component of marijuana, and *dl*-Δ^8-tetrahydrocannabinol (an oil), which is the racemate of a minor active component of marijuana. Our results were obtained with these two racemates (*9*), and they indicate that both *dl*-Δ^8- and *dl*-Δ^9-tetrahydrocannabinols (hereafter termed Δ^8-THC and Δ^9-THC) are potent psychotropic agents producing pronounced behavioral aberrations.

Behavioral effects of these tetrahydrocannabinols were measured with operant conditioning techniques. The conditioning procedures used were: (i) continuous avoidance in squirrel monkeys (*10*), (ii) shock titration in squirrel monkeys (*11*), and (iii) delayed matching in rhesus monkeys (*12*). Several doses of both Δ^8- and Δ^9-THC were tested in from 4 to 11 subjects in each procedure. Generally, animals were drugged only once every 2 weeks, and no animal was ever drugged more frequently than once a week. For injection, Δ^9-THC was prepared by suspending it in 5 percent gum arabic alone or in gum arabic after initial mixing in three drops of glycerin or sesame oil; Δ^8-THC was suspended in gum arabic, but only after much levigation with glycerin or sesame oil. Before being prepared for injection the drugs were stored at −70°C, and care was taken that samples had minimum exposure to air (*13*).

The effects of Δ^9-THC in the continuous avoidance procedure are difficult to describe. Intraperitoneal doses of 4 or 8 mg/kg decreased the response rate to about 50 percent of the individual subject's own control rates. However, as the dose was further increased to 16, 32, and 64 mg/kg, the animals were frequently stimulated. They responded at about twice (200 percent) their control rates. This increased lever-pressing was not seen in all animals perhaps for reasons given below.

Observed changes in the general behavior of squirrel monkeys given Δ^9-THC are more relevant than changes in lever-pressing in this case. Monkeys given 4 or 8 mg/kg of Δ^9-THC sat quietly near the levers with head down and seemingly peered at the lower part of the box. Dosages of 16 mg/kg stimulated or excited the monkeys and caused them to walk about the box, apparently looking at something the experimenters did not see, or to crouch and move their heads from side to side and up and down as if watching some moving object. Some animals had a blank expression and gazed into space. We assumed that the animals had visual hallucinations, but the extent to which THC affects the oculomotor or other visual systems is unknown. In some monkeys when the dose of Δ^9-THC was 16 mg/kg, and in all monkeys given 32 or 64 mg/kg, this apparent hallucinatory reaction was more obvious. Monkeys moved quickly about the box, looked above and behind themselves, seemed to be in a state of panic (*14*), and appeared to fight with imaginary objects; their arms would swing rapidly through the air and they would attempt to grasp objects that were not there. These movements were rapid and associated with fine hand tremors. The fighting and swatting movements appeared well coordinated. However, it was impossible to determine whether some of these movements were completely voluntary. The animals tended to maintain one or two limbs in an unusual position; for example, one hind leg flexed against the abdomen. The subjects also tended to look intently at their widely opened hands; then one of the hands (sometimes both) was partly closed with palm up, and it was then held near the chest for several hours. The onset of the effect after small doses (4 to 8 mg/kg) of the drug was gradual, and about 1 hour was required after injection until behavior was clearly altered. Higher doses were active within 20 minutes. The stimulant phase of the drug action persisted about 3 hours and was followed by a period of depression (the animals assumed a crouched position and remained almost motionless). This depression lasted for 1 to 2 days and occasionally for a week. Nine subjects died after being severely depressed for 24 to 72 hours (*15*).

At intraperitoneal doses of 2, 4, and 8 mg/kg, Δ^8-THC increased the rate of lever-pressing in monkeys in the avoidance procedure. In contrast to Δ^9-THC, Δ^8-THC did not decrease lever-pressing at lower doses, and the stimulation produced by higher doses of Δ^8-THC was not followed by depression or death. The same type of bizarre effects were produced by both drugs, except that the effects of Δ^8-THC were somewhat more pronounced and typically were

associated with increased locomotor activity.

A second type of shock avoidance procedure (*11*), shock titration, was used to assess the possible analgesic effects of the drugs. There was a tendency for both drugs to increase shock tolerance at intraperitoneal dosages of 4 to 8 mg/kg, but it is doubtful that this represents a purely analgesic effect. The subjects occasionally allowed the shock intensity to increase 1.0 ma above control values but then responded to decrease the shock level. Thus, the drugs did not have general sedative or analgesic effects but rather made the subject's performance more variable. Changes in gross behavior were not observable because the subjects were restrained in the chair. However, the animals did frequently seem to stare into space and be unaware of the experimenter's presence (*16*).

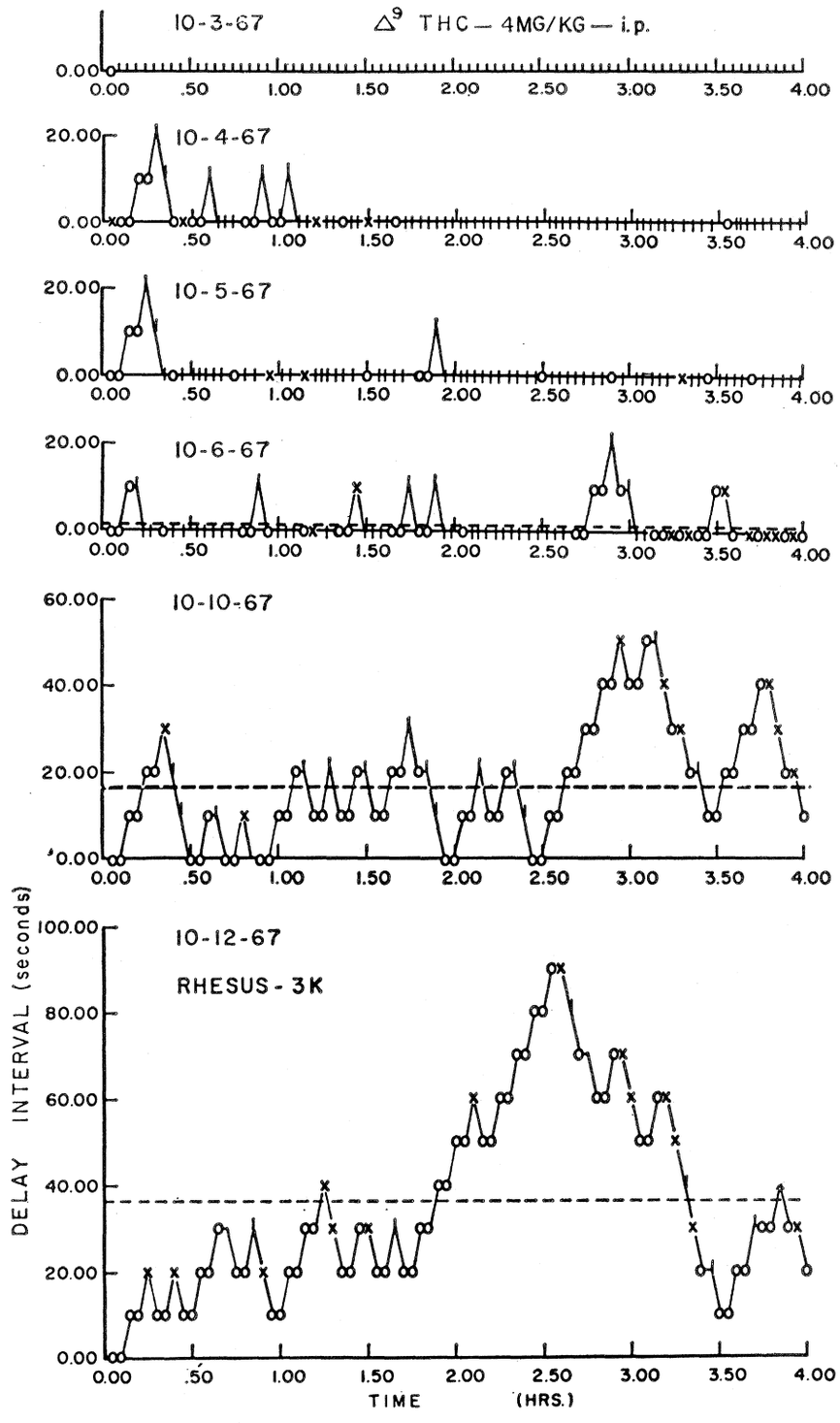

Fig. 1. Long-term effect of one intraperitoneal dose of 4 mg of Δ^9-THC per kilogram of body weight on a rhesus monkey in the delayed matching procedure. Open circles, correct matching responses; *X*, an incorrect match; short vertical lines, failures to respond to the sample; dashed horizontal lines, average level of performance during that session.

The disruption of behavior produced by these drugs was seen most objectively in rhesus monkeys when we used a delayed matching procedure (*12*) (Fig. 1). On 3 October 1967 a subject received a single intraperitoneal dose of 4 mg of Δ^9-THC per kilogram of body weight 15 minutes before the session started and subsequently made only one correct matching response (as shown in the top record). After the first trial the animal stopped responding. At the end of the session, the subject was returned to its home cage where it did not react as actively or alertly as normal. However, the animal ate its food normally, indicating that the drug's effect on delayed matching was not due to anorexia. Retesting during the following days indicated that the behavior of the subject was severely disrupted for at least 4 days after administration of one dose. On the 7th day the subject's performance began to improve and was normal 9 days after injection (bottom record).

Doses of 2 and 1 mg of Δ^9-THC per kilogram of body weight had effects similar to those shown in records two and four, respectively (Fig. 1). At dosages of 0.25 mg/kg the effect was similar to that shown in the fifth record (10 October 1967; Fig. 1). The duration of action of these lower doses was more than 4 hours but less than 3 days. The effects of both drugs were similar, except that the effects of high doses of Δ^8-THC lasted less than 3 days.

In general, the results show that both drugs profoundly affect the behavior of monkeys and cause stimulation, depression, apparent hallucinations, and the loss of ability or motivation to perform complex tasks. We used the racemic compounds in this study; but work in our laboratory with synthetic levorotatory Δ^8- and Δ^9-THC (*17*) indicates that these isomers have essentially the same activities as the racemates but are more potent. We conclude that both *l*-Δ^8-THC and *l*-Δ^9-THC are potent psychotropic drugs. On the basis of our data, obtained after

parenteral administration, anyone conducting clinical research with these drugs should consider the possibility of long-term effects.

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8. K. E. Fahrenholtz, M. Lurie, R. W. Kierstead, *J. Amer. Chem. Soc.* **88**, 2079 (1966); *ibid.*, **89**, 5934 (1967).
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12. C. L. Scheckel, *J. Comp. Physiol. Psychol.* **59**, 415 (1965). Food-deprived subjects were individually placed in a large box containing three levers. Above each lever was a translucent disc (2.54 cm diameter) that could be illuminated with a red, green, or white light. Normally the discs were white, but every 3 minutes a sample stimulus was presented at the center disc. The sample was either a red or green light which remained on for a maximum of 10 seconds. The subjects were trained to press the lever under the sample, and this response started a delay interval during which all lights were turned off. After the delay, only the side lights came on; one side was red and the other green. The animal's task was to press the lever under the side disc that was the same color as the sample had been (match the sample). Correct matching responses were rewarded with a 190-mg banana pellet. Incorrect responses terminated the trial with no reward. The duration of the delay interval (the time that the subject had to "remember" the color of the sample) varied as a function of the animal's performance. At the start of each 4-hour session the delay was set at 0 seconds, but every time the subject made correct matching responses on two consecutive trials at one delay, the delay presented during the next trial was automatically increased by 10 seconds. Whenever a subject failed to match correctly or failed to respond to the sample, the delay interval in the next trial decreased 10 seconds. Thus, as the subject performed correctly the problem became more difficult, but errors or a failure to respond to the sample made the problem somewhat easier.
13. J. Sullivan and E. Boff, in preparation.
14. The hallucinations and panic states produced by racemic Δ^9-THC injected into monkeys correlate well with the effects of high doses of *l*-Δ^9-THC and marijuana smoked by man [H. Isbell, C. W. Gorodetzsky, D. Jasinski, U. Claussen, F. V. Spulak, F. Korte, *Psychopharmacologia* **11**, 184 (1967); M. H. Keeler, *Amer. J. Psychiat.* **124**, 674 (1967)].
15. Death occurred in two of five subjects that received 16 mg of Δ^9-THC per kilogram, in all five subjects at dosages of 32 mg/kg and in two of four subjects at dosages of 64 mg/kg. The exact cause of death is unknown, but was probably due to a combination of respiratory depression, heat loss, and decreased food and water intake.
16. All subjects in this procedure vomited within 1 hour after receiving Δ^9-THC, but Δ^8-THC did not cause emesis in any subject.
17. Supplied by Dr. T. Petrzilka, Eidgenosse Technischen Hochschule, Zürich.
18. We thank Drs. K. E. Fahrenholtz and R. W. Kierstead for making available the racemic drugs, J. W. Sullivan for technical assistance, and Dr. A. Brossi for encouragement.

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Alcohol Preference in the Rat: Reduction Following Depletion of Brain Serotonin

Abstract. *Preference for ethyl alcohol was significantly reduced or totally abolished in rats given orally* p-*chlorophenylalanine, a tryptophan hydroxylase inhibitor that selectively depletes brain serotonin. Some aversion to alcohol was observed while* p-*chlorophenylalanine was administered, but the rats' rejection of alcohol was even more marked after the drug was discontinued. Oral administration of* α-*methyl*-p-*tyrosine, a tyrosine hydroxylase inhibitor that depletes brain catecholamines, slightly reduced selection of alcohol, but preference returned to normal as soon as* α-*methyl*-p-*tyrosine was terminated.*

Repeated microinfusions of minute amounts of alcohol into the cerebral ventricles of unrestrained rats produce a dose-dependent preference for alcohol (*1*). This observation has led to the hypothesis that metabolic systems, in the limbic-forebrain structures lining the walls of the ventricles, are directly affected by the presence of alcohol, and that the biochemical state of these systems may underlie the aberrant drinking patterns observed in the chronic alcoholic (*2*).

To determine whether a neurochemical imbalance would either trigger or suppress an animal's preference for alcohol, compounds were chronically administered that alter endogenous substances in the brainstem regions involved in drinking and emotional behavior. In the experiment we report, the concentrations of monoamines were selectively lowered by either *p*-chlorophenylalanine (*p*CPA), a tryptophan hydroxylase inhibitor that substantially depletes brain serotonin (*3*), or α-methly-*p*-tyrosine (αM*p*T), which has a potent depletive action on brain catecholamines (*4*).

Adult male hooded rats of the Long-Evans strain, divided into three groups of six each, were maintained in individual cages and freely given Wayne Lab Blox throughout. Stable preference-aversion functions for alcohol (*5*) were obtained for each animal by offering water and a solution of alcohol simultaneously according to the two-choice, three-bottle, random-rotation method (*6*). Concentrations of alcohol were increased daily in the following sequence: 3, 4, 5, 6, 7, 9, 12, 15, 20, 25, and 30 percent. Thus alcohol preferences were validly measured over a broad range of concentrations for elimination of the drawbacks of a single-concentration method (*7*). The 11-day self-selection sequence was repeated three times: before, during, and after administration of saline vehicle to the first group, αM*p*T to the second, and *p*CPA to the third.

Each day of the 11-day drug period the compounds were given by the intragastric route and, to minimize the trauma of intubation, each rat was lightly sedated in an ether–ethyl chloride vapor chamber for 40 to 50 seconds before insertion of the esophageal tube. Alpha-methyl-*p*-tyrosine in normal