# EFFECTS OF $\Delta^9$-TETRAHYDROCANNABINOL AND MESCALINE ON SELF-STIMULATION

P. T. BAILEY and S. N. PRADHAN
Department of Pharmacology, Howard University, College of Medicine, Washington D.C.

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**Summary**—The effects of two psychotomimetic drugs, $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) and mescaline on intrahypothalamic self-stimulation behavior were investigated to test their action on the central reward system. Rats were trained to press a lever to receive intracranial electric stimulation through a set of stereotaxically implanted bipolar electrodes in the posterior lateral hypothalamus. Both $\Delta^9$-THC (1–25 mg/kg in 4% Tween in saline) and mescaline (6·3–25 mg/kg) injected i.p. caused a decrease in self-stimulation responding. A rough dose–response relation was observed with both the drugs at these doses. Following repeated injection of a particular daily dose, tolerance developed to both these drugs within 9 days. However, no cross-tolerance was observed between these two drugs in this schedule.

OPERANT behavior of a subject can be controlled by reinforcements that may be "positive" (i.e. rewarding) or "negative" (i.e. punishing). The investigations of Olds and coworkers (see OLDS, 1958, 1962) have demonstrated areas in the brain, electrical stimulation of many of which is rewarding and as such induces positive motivational effects, while that of others are punishing and shows negative motivational effects. Of all the positively reinforcing brain areas, the median forebrain bundle—lateral hypothalamic area produces the highest self-stimulation rates, has the lowest stimulation intensity threshold and is the most resistant to satiation effects (OLDS and OLDS, 1964). Intrahypothalamic self-stimulation responding thus appears not only to provide a measure of centrally mediated positive reinforcement, but also an index of motivational effect.

Some drugs that alter mood and relieve tension have a higher abuse liability than others. One obvious reason is that some of these drugs have been found pleasurable or relaxing by some individuals, although not by others (JAFFEE, 1965). These drugs appear to induce a motivating and positively reinforcing effect in addicts. Testing their effects on intracranial self-stimulation responding may further elucidate their behavioral action. Effects of some of these drugs have been investigated on the central reward system. Examples of these are the studies on the effects of amphetamine (STEIN, 1964a,b) cocaine (CROW, 1970) and morphine and barbiturates (OLDS and TRAVIS, 1960) on self-stimulation. This report describes the effects of $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC, a potent constituent of marihuana) and mescaline (an alkaloid from Peyote) on self-stimulation responding and development of tolerance to these drugs.

## METHODS

The method and procedure of self-stimulation schedule and of testing drug effects on it have been described in detail in an earlier publication (PRADHAN and BOWLING, 1971) and will only be summarized here.

Male albino rats (Wistar-derived Walter Reed strain) with initial body weight of 250–350 g were used in this experiment. A set of bipolar stainless steel electrodes was stereotaxically implanted in the posterior lateral hypothalamus of each of 13 rats under pentobarbital (50 mg/kg i.p.) anesthesia. The stereotaxic coordinates were 3·5 mm posterior to the bregma, 0·75 mm lateral to the midline and 9 mm in depth from the top of the skull.

After recovery from surgery, the rats were trained to press a lever in a Skinner box for reinforcement with intracranial electric stimulation. Each lever press provided the stimulus train of 0·4 sec duration, stimulus being a sine wave of 60 Hz. The current intensity (as rms) remained constant at slightly above threshold level throughout the sessions. Responses were recorded every 10 min during a 60–90 min session.

Rats were subjected to a daily session 5–6 days a week. When the performance of each rat in this schedule reached a stable level after about two months of training, treatment with drugs was started. A dose of $\Delta^9$-THC (1, 5, 10, 15 and 20 mg/kg in 4% Tween in saline) or mescaline hydrochloride (6·3, 12·5, 18 and 25 mg/kg in normal saline) was injected i.p. in increasing sequence at an interval of at least 3 days. Normal saline (0·2–0·4 ml) was given i.p. on 2 days before each drug day. The vehicle 4% Tween in saline was injected as an additional control and taken as 0 dose of $\Delta^9$-THC. A dose of a drug was injected only when the control data from two successive daily sessions reached the previous baseline level and were within 10% of their average. Injection was given immediately before the session, unless otherwise mentioned. For inducing tolerance, a dose of a drug was injected daily.

The data from the drug sessions were reported as the per cent change from the corresponding control rate; the latter was calculated as the mean of the data from the two control sessions preceding a drug session. Such data from several rats at a particular dose level were pooled to calculate the mean and the standard error of the mean (S.E.). Student's $t$ was calculated to evaluate the statistical significance of the change from the control.

## RESULTS

The responding rate of the rats used in this study varied from 750 to 4300 per hour. Their responding was usually decreased following administration of either $\Delta^9$-THC or mescaline. The effects of the drugs at the doses used did not show any relationship to the baseline rates of the animals except in some rats that showed slight increase of responding at low doses. Figure 1 shows the time-course of effects of 5 doses (1–20 mg/kg) of $\Delta^9$-THC and the vehicle, 4% Tween in saline (0 dose). Table 1 summarizes the overall effects of these doses on 13 rats during 90-min sessions. It appears that Tween itself caused a slight increase in responding, although not significant. Barring an initial increase at low doses in some animals, $\Delta^9$-THC at the doses used appeared to cause an overall decrease in responding. One rat failed to show any effect at the lower doses used and caused an increase in responding at the higher doses. The effect was manifested within 10–20 min after the drug administration. The magnitude of decrease and the number of animals showing such effect were found to increase roughly with the dose; the effects of 1 and 5 mg/kg doses appeared to be close to each other, and so also those of 10, 15 and 20 mg/kg doses. At the end of 90-min sessions the effect still persisted, and was rather at its maximum intensity at 10–20 mg/kg doses. In 7 rats tested at different intervals after injection of 10 mg/kg doses such depression was found to last for 4–8 hr.

The time-course of the effect of mescaline (6·3–25 mg/kg) on self-stimulation during 60-min sessions in 10 rats are illustrated in Fig. 2. The self-stimulation responding was

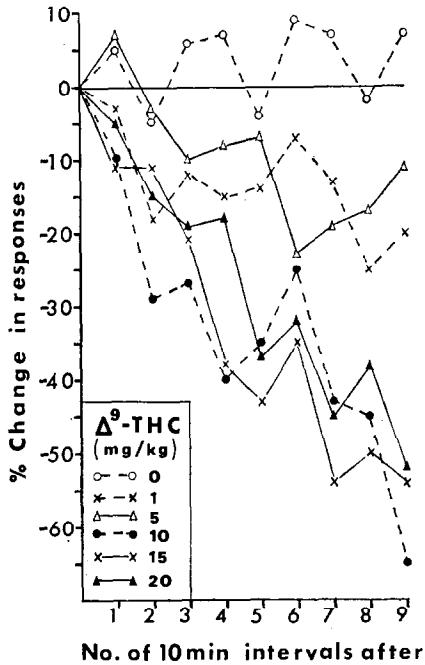


FIG. 1. Time-course of effects of various doses of $\Delta^9$-THC together with 4% Tween in saline (0 dose) on self-stimulation behavior during 90-min sessions. Each point represents an average of data from 13 rats, one from each animal.

TABLE 1. EFFECTS OF VARIOUS DOSES OF $\Delta^9$-THC ON SELF-STIMULATION

| Dose* (mg/kg) | No.† of rats showing effect‡ | | | Overall effect (% change from control) |
|---|---|---|---|---|
| | + | 0 | − | Mean±S.E. |
| 0 | 3 | 8 | 2 | 6±5 |
| 1 | 2 | 5 | 6 | −16±9§ |
| 5 | 1 | 4 | 8 | −17±7§ |
| 10 | 1 | 2 | 10 | −36±9‖ |
| 15 | 1 | 0 | 12 | −35±8‖ |
| 20 | 1 | 1 | 11 | −32±9‖ |

*4% Tween in saline was used as vehicle and was injected as zero dose.
†A total of 13 rats were used.
‡+ Increase, 0 no change, − decrease of responding.
§Significantly different ($P<0{\cdot}05$) from effect of Tween.
‖Significantly different ($P<0{\cdot}01$) from effect of Tween.

decreased by the various doses of mescaline showing a dose–response relationship. The onset of drug action occurred within 10 min and the peak effect within 30–40 min. The overall effect of mescaline computed from the total responses during the 60-min sessions was a decrease in the rate of responding in the majority of the sessions at these doses; only 3 rats showed a slight increase at low doses (Table 2). Decrease of responding increased with the dose. Tested over periods of 120–150 min, 3 out of 5 rats showed a biphasic response, response rate being increased above normal after a period of initial decrease.

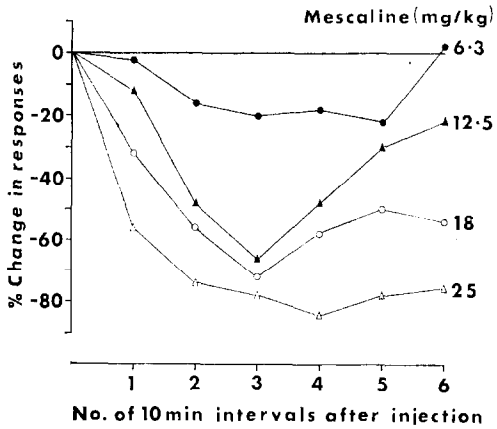


FIG. 2. Time-course of effects of various doses of mescaline on self-stimulation behavior during 60-min sessions. Each point represents an average of data from 10 rats, one from each animal.

TABLE 2. EFFECTS OF MESCALINE ON SELF-STIMULATION IN RATS

| Dose (mg/kg) | No.* of rats showing Increase of responding | No.* of rats showing Decrease of responding | Overall effect (% change from control) Mean±S.E. |
|---|---|---|---|
| 6·3 | 3 | 7 | −11±8 |
| 12·5 | 1 | 9 | −44±9† |
| 18 | 0 | 10 | −54±8† |
| 25 | 0 | 10 | −70±7† |

*Total of 10 rats.
†$P<0{\cdot}01$.

Possibility of development of tolerance to $\Delta^9$-THC (10–20 mg/kg) and mescaline (25 mg/kg) was investigated by injecting a daily dose of the drug for a number of days and recording its effect on responding.

Figure 3 presents the responses of 4 rats under the self-stimulation schedule before, during and after the treatment with the same daily doses of $\Delta^9$-THC. The response in these animals was decreased to a minimum after 1–3 days. Then the rate gradually returned very close to or above the baseline level within 7 days, showing the development of tolerance in all 4 rats.

Cross-tolerance between $\Delta^9$-THC and mescaline was studied in two $\Delta^9$-THC-tolerant rats (Fig. 4). For each rat a test dose of each of mescaline and $\Delta^9$-THC was selected that previously caused an adequate decrease in responding. At first the test dose of mescaline was administered followed by that of $\Delta^9$-THC after 2 weeks. After an interval of 5 days, the same dose of $\Delta^9$-THC was injected daily. Tolerance developed between the 5th and the 9th days in these rats. In both these rats, mescaline again produced a decrease in responding, thus showing a lack of cross-tolerance to mescaline in $\Delta^9$-THC-tolerant rats.

In order to produce tolerance to mescaline 4 rats were injected daily with a dose of 25 mg/kg for a period of 11 days. The first injection produced a marked decrease in response

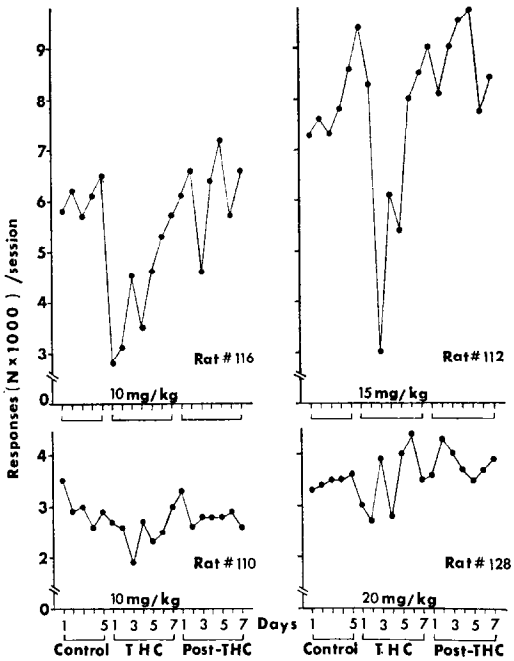


FIG. 3. Development of tolerance to various doses of $\Delta^9$-THC in 4 rats. Total numbers of responses during successive control, drug, and post-drug sessions are recorded. N represents the number in the ordinate. Saline was injected during the control sessions.

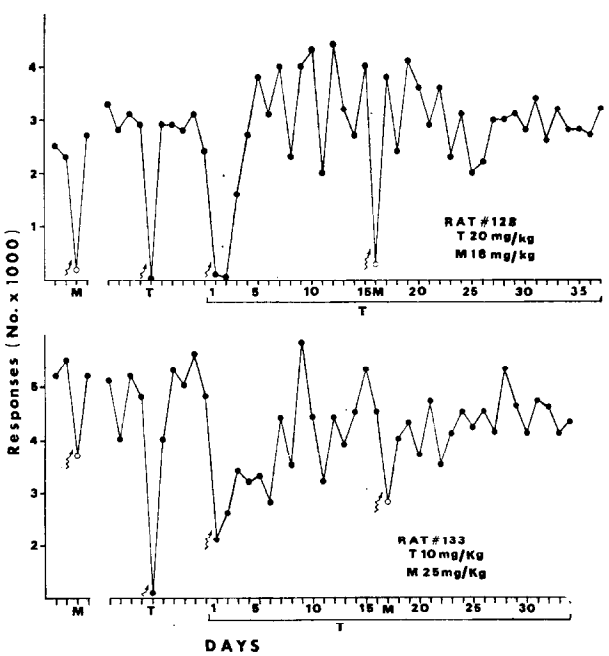


FIG. 4. Lack of cross-tolerance to mescaline (M) in $\Delta^9$-THC (T)-tolerant rats. Test doses of M and T were given initially at the interval of 2 weeks. After 5 days, T was injected daily. A dose of M was injected when tolerance to T developed after 5–9th injection. Note the increase of responding in rat #128 after development of tolerance.

which gradually returned to or increased slightly above the baseline rate within 7 days. Figure 5 presents such development of tolerance to mescaline in two representative animals.

To investigate the development of cross-tolerance between mescaline and $\Delta^9$-THC, 4 rats were injected with a test dose, each of $\Delta^9$-THC (10 mg/kg) and mescaline (18 mg/kg) at intervals of 1–2 weeks, as in previous cross-tolerance experiment. Five days after the latter injection, the same dose of mescaline was injected daily. When the rats became tolerant to mescaline, the dose of $\Delta^9$-THC was administered again producing a decrease in response and demonstrating a lack of cross-tolerance to $\Delta^9$-THC in mescaline-tolerant rats. Figure 6 illustrates effects of these drugs in 2 representative rats.

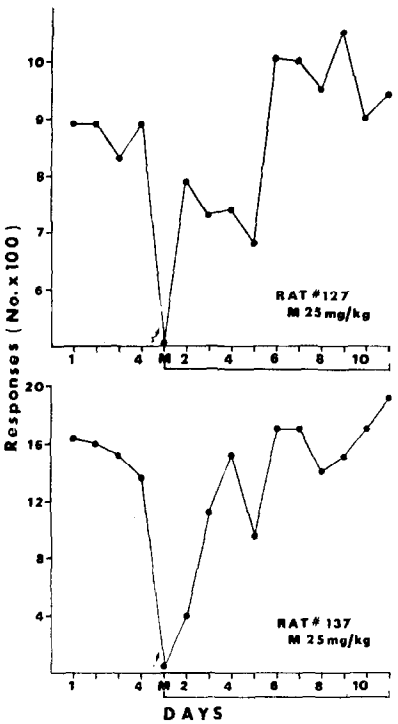


FIG. 5.

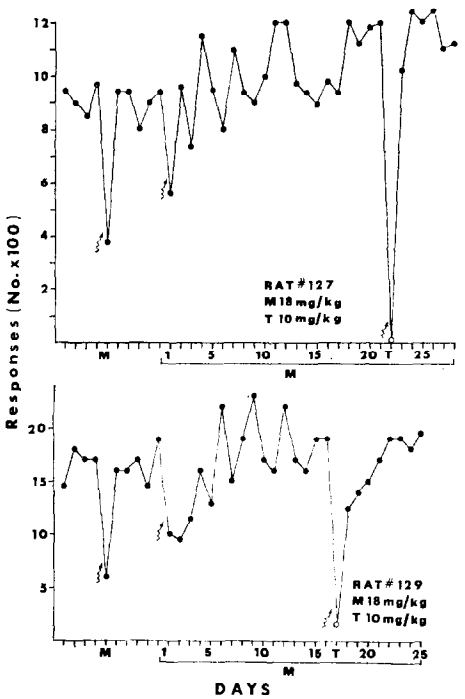


FIG. 6.

FIG. 5. Development of tolerance to 25 mg/kg of mescaline (M). Note the increase of response in rat #127 after development of tolerance.

FIG. 6. Lack of cross-tolerance to $\Delta^9$-THC (T) in mescaline (M)-tolerant rats. Procedure was more or less similar to that illustrated in Figure 4. Note the increase of responding of both the rats in several sessions following development of tolerance.

## DISCUSSION

Effects of abused drugs on self-stimulation behavior appear to vary with the nature of their central action. Thus, self-stimulation responding was shown to be enhanced by the stimulant drugs, e.g. amphetamine (STEIN, 1964a,b) and cocaine (CROW, 1970). On the other hand, morphine inhibited intrahypothalamic self-stimulation, whereas pentobarbital and meprobamate augmented the same in some animals and inhibited in others at the doses

used (OLDS and TRAVIS, 1960). In the present experiment, both $\Delta^9$-THC and mescaline decreased self-stimulation responding, as in the case of morphine. Such decrease in responding is also consistent with the effects of $\Delta^9$-THC and other marihuana derivatives (BOYD, HUTCHINSON, GARDNER and MERRITT, 1963; CARLINI, 1968; SILVA, CARLINI, CLAUSEN and KORTE, 1968; MCMILLAN, HARRIS, FRANKENHEIM and KENNEDY, 1970; see PRADHAN and BAILEY, 1972) as well as those of mescaline (FREEDMAN, AGHAJANIAN, ORNITZ and ROSNER, 1958; SMYTHIES *et al.*, 1966, 1967; SILVA *et al.*, 1968; APPEL and FREEDMAN, 1968) on other reinforced behaviors.

A difference in the sensitivity in case of both the drugs was observed in a few rats that showed slight increase in responding, especially at low doses. However, with the increase in dose, the responding was decreased. Some rats showed a biphasic response. However, this biphasic effect was not always observed, nor was there any marked "excitatory" effect at the doses used, as reported by SMYTHIES *et al.* (1966) on conditioned avoidance response (CAR) at high doses of mescaline.

Development of tolerance to marihuana derivatives (CARLINI, 1968; SILVA *et al.*, 1968; MCMILLAN *et al.*, 1970; see PRADHAN and BAILEY, 1972) as well as to mescaline (SMYTHIES *et al.*, 1966; SILVA *et al.*, 1968; APPEL and FREEDMAN, 1968; FREEDMAN *et al.*, 1958) has been reported in a variety of schedules. Development of tolerance to inhibition of self-stimulation responding by $\Delta^9$-THC and mescaline in the present investigation adds to the list. In the present study tolerance was shown to develop to the effects of 10–20 mg/kg of $\Delta^9$-THC and 18 mg/kg of mescaline. It is possible to develop tolerance at lower doses of these drugs, as already been shown in other behavioral schedules at 1 and 5 mg/kg of $\Delta^9$-THC (BAILEY, PRADHAN and GHOSH, 1971) and at 10 mg/kg of mescaline (FREEDMAN *et al.*, 1958; APPEL and FREEDMAN, 1968). Mechanisms of tolerance to these drugs are not clear. The tolerance to a drug may be induced at the level of their metabolism including their absorption and excretion and/or at the cellular level causing the target tissues to be less responsive to drug action. However, information regarding such mechanisms with respect to these drugs is not available at present.

In the case of mescaline that showed a biphasic effect on CAR in rats, its successive daily doses induced tolerance to its "inhibitory" effect, but the "excitatory" effect was increased (SMYTHIES *et al.*, 1966). In the self-stimulation schedule, such phenomena was observed in a few rats that showed an increase of responding in the case of both mescaline (rat #127 in Figs. 5 and 6 and rat #129 in Fig. 5) and $\Delta^9$-THC (rat #128 in Figs. 3 and 4) following development of tolerance to their depressant effects.

The present study also demonstrates a lack of cross-tolerance between $\Delta^9$-THC and mescaline in self-stimulation schedule. Similar lack of cross-tolerance between these drugs has been reported by SILVA *et al.* (1968) in rope-climbing and bar-pressing (for water reinforcement) behaviors in rats.

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