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Learned Behavioral Tolerance to Marihuana in Rats[1]

BROOKS CARDER AND JAMES OLSON

Department of Psychology, University of California, Los Angeles, California 90024

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CARDER, B. AND J. OLSON. *Learned behavioral tolerance to marihuana in rats.* PHARMAC. BIOCHEM. BEHAV. 1(1) 73–76, 1973.–Rats were trained to press a lever for food reinforcement in one study and water reinforcement in a second. Rats which received marihuana extract each day before behavioral testing showed an impairment of responding on the first day of drug application, but developed behavioral tolerance to the drug by the sixth day of drug application. Rats which received equal doses of marihuana after each session, rather than before, over the same period, showed little or no evidence of behavioral tolerance when the drug was administered before testing. This result was interpreted to indicate that the development of behavioral tolerance to marihuana involves a learning process.

Marihuana Behavioral tolerance Learning

THERE have now been a number of demonstrations that repeated administration of *Cannibis* preparations leads to the development of behavioral tolerance in animals [2, 6, 9, 11]. Perhaps most striking are the data of McMillan and his associates [6, 9], indicating that in pigeons and rats the rate of development of behavioral tolerance to 1-Δ^9 THC, and the ultimate level which the tolerance reaches, are comparable to the development of tolerance to morphine. After a series of increasing doses, animals efficiently performed operant responses following a dose of THC that would anesthetize an inexperienced animal for several hr.

Most of the demonstrations of behavioral tolerance to *Cannabis* preparations, however, have involved a procedure in which a subject is repeatedly dosed with *Cannabis* and then behaviorally tested after each dose. The development of tolerance in these experiments could have been a result of the operation of either of two types of mechanisms: (1) repeated application of the drug could produce simple physiological alterations, resulting in tolerance; and, (2) alternatively the animal may have learned to cope with the effect of the drug in some way, indicating a rather more complex physiological basis for the development of tolerance. It is possible to discriminate between these two alternatives experimentally. Chen [4] devised a procedure in which one group of animals receives daily drug doses followed by behavioral tests, as in the usual behavioral tolerance experiment, while a second group receives a behavioral test followed by a drug dose each day. Both groups receive equivalent physiological exposure to the drug, but the second group has no opportunity to learn to cope with the drug in the behavioral test situation. The drug-before group develops and displays behavioral tolerance over the course of several days. If this tolerance is a result of a simple, drug induced physiological change then the drug-after group should have developed tolerance also and display it when given the drug before testing. If tolerance in the drug-before group has resulted from learning to cope with the drug, the drug-after group would not have developed tolerance, and should not display it when drugged before testing. Learned tolerance has been shown to develop to a number of drugs including barbiturates and alcohol [7].

EXPERIMENT 1

Previous work from our laboratory has indicated that the effects of marihuana are crucially dependent upon the animals' past experiences. For example, marihuana increases shock induced aggression in rats, but only in rats that have not been exposed previously to marihuana or to the agression test situation [3]. These data suggest that learning plays a potent role in the reaction of an animal to marihuana, and have prompted us to investigate the role of learning in the development of behavioral tolerance to marihuana.

Method

Twenty male Sprague-Dawley rats from the UCLA Department of Zoology vivarium were deprived of food and maintained at 80% of their free feeding weight. All were trained to press a lever for 45 mg food pellets on a continuous reinforcement schedule in a standard Lehigh Valley Electronics operant conditioning chamber. When the response was established, each animal received seven daily

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15-min sessions in the lever press apparatus. Animals were then divided into two groups of 10, equated for the number of presses on the last three training days.

For the next six daily sessions, each animal received an injection one hr before the test session and another injection 45 min after the test session. Animals in Group THC-before received an intraperitoneal injection of marihuana distillate (NIMH) diluted in propylene glycol to yield a suspension with a potency of 4.0 mg Δ^9-THC/ml. The dose for each animal was 3.0 mg Δ^9-THC/kg. This dose of marihuana produced no striking change in the behavior of the animals outside the test situation. The drugged animals appeared slightly lethargic. After testing, these animals received an equal volume of propylene glycol. Animals in Group THC-after received the propylene glycol injection before each test session and the marihuana injection after.

On the next three days of testing, half of each group had the order of injections reversed, while the other half were continued as before.

Results and Discussion

Figure 1 presents the average number of presses in each 15-min test session for the two groups for the seven predrug days, six days over which behavioral tolerance developed, and three days on which the order of drug administration was reversed for some animals. The administration of marihuana before testing produced a statistically reliable decrease in responding (compared to the responding of the animals that received the drug after testing) on tolerance Day 1 (t=4.04, df=18, $p<0.01$ two-tailed) and tolerance Day 2 (t=2.82, df=18, $p<0.02$). The THC-before group responded as much as the THC-after group on the sixth tolerance day.

On the three reversal days the group shifted from THC-after to THC-before (labeled THC-after/before in the figure) responded much less than the other three groups. A one-way analysis of variance of the data from the first reversal day indicated a significant effect of drug treatment (F=4.68, df=3/16, $p<0.05$). Over the three reversal days there was no apparent improvement in the performance of the animals shifted from THC-after to THC-before.

It is clear from the figure that during the initial phase of drug administration there is a marked decline in responding of the THC-after group. In fact, the THC-before group does not improve, but eventually equals the THC-after group because of the decline in the latter's performance. This decline is apparently related to the introduction of drug administration since the predrug baseline was quite stable. There are several possible reasons for this decline: (1) The half-life of THC and its metabolites in the blood is apparently quite long [10]. The steady decline in performance may therefore represent drug accumulation. If this were the case, however, the THC-after group would have been performing under the influence of the drug and it would then be difficult to explain why these animals failed to demonstrate tolerance when given the drug before testing. (2) It has been demonstrated that the administration of marihuana following the consumption of sucrose, in doses as low as 1.0 mg THC/kg, will produce an aversion to the sucrose, so that less is consumed on a subsequent test [5]. Thus the THC-after group may have developed an aversion to the food pellets delivered in the experimental situation and responded less. This explanation, however, fails to account for the observation, on reversal testing, that the THC-before animals have a

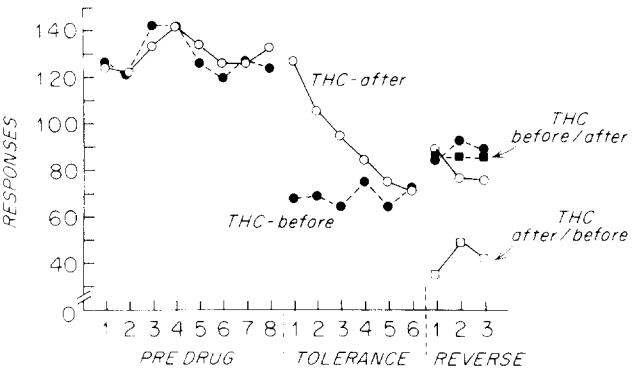

FIG. 1. Lever pressing for food as a function of marihuana administration. Between-group differences are statistically reliable on tolerance Days 1, 2, and 3 ($p<0.05$ by t-test). Analysis of variance indicated a statistically reliable interaction on reverse Day 1 ($p<0.05$).

tolerance to the drug that the THC-after animals have failed to acquire. (3) It has been demonstrated that single daily doses of 4 mg THC/kg, given intraperitoneally, produce a persistent reduction of food intake [8]. The simplest way to account for the present data is to assume that both groups became less hungry as a result of the daily administration of marihuana, and therefore responded less for food in the test situation. Thus the development of behavioral tolerance in the THC-before group would be superimposed on a generalized suppression of appetite in both groups.

In any case, it is apparent that the administration of marihuana following behavioral testing does not lead to substantial tolerance in the lever press situation. Animals shifted from THC-after to THC-before perform quite poorly when compared to unshifted animals or to animals shifted from THC-before to THC-after. Thus the data indicate that some form of learning plays a significant role in the development of behavioral tolerance to marihuana.

EXPERIMENT 2

The first experiment indicated the importance of a learning process in the development of tolerance to marihuana. Interpretation of the data, however, was made difficult by a gradual decline in the performance of animals receiving the drug following behavioral testing. In addition, the first study employed relatively low doses of marihuana. It might be argued that learning plays a role in the development of tolerance to low but not to high doses of marihuana. In order to confirm and extend the results of the first study, a second experiment was carried out. Animals were run only every second day to minimize the possibility of drug accumulation. Water reinforcement was employed to obviate problems of bait shyness and appetite suppression. High doses of marihuana were administered orally to prevent the marihuana produced irritation reported to result from intraperitoneal administration [8].

Method

Twenty female Sprague-Dawley rats were individually housed and deprived of water on a 24-hr schedule. This involved a 15-min exposure to water each day in the home

cage. On experimental days this exposure was given within one hr of the test session. Lever press training and testing were carried out in a standard Gerbrands operant conditioning chamber. Each press refilled a dipper with 0.1 ml of water.

When the response was established, the animals received a 5-min lever press session every second day throughout the experiment. When a stable baseline of responding was achieved, drug administration was begun. A flexible vinyl tube was inserted into the mouth and passed down the esophagus into the stomach. A propylene glycol marihuana suspension or the propylene glycol placebo was injected into the tube and washed down with 1 ml of water. Drugs were administered 1 hr before testing and within 15 min after testing. As before, in any test session animals received either marihuana or propylene glycol before testing and the alternate preparation following testing.

In a preliminary series of experiments, an attempt was made to replicate the first experiment using an oral marihuana dose of 8 mg THC/kg. Analysis of the data revealed that this dose failed to suppress responding significantly when given before testing. In addition, the behavioral baseline was quite variable at times. Therefore, while the general pattern of results was not inconsistent with the result of the first experiment, the data are of little value. For this reason only data of the final portion of the experiment, with much higher marihuana doses, will be discussed in detail. We will describe all of the experimental procedures applied to the animals, however, so that their history of training and drug experience can be evaluated.

One group of 10 rats (open circles in Fig. 2) received marihuana (8 mg THC/kg) before testing for five sessions, marihuana after testing for six, marihuana before testing for three more, and marihuana after testing (in increasing doses) for the remainder of the experiment until the final reversal.

The other group (filled circles in Fig. 2) received marihuana after testing for five sessions, marihuana before testing for nine sessions, marihuana after testing for six, and marihuana before testing (in increasing doses) for the remainder of the experiment until the final reversal.

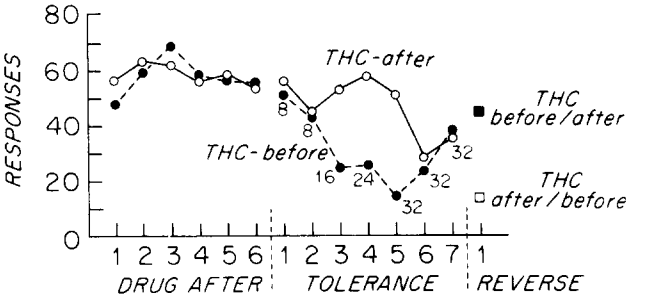

FIG. 2. Lever pressing for water as a function of marihuana administration. Between-group differences are statistically reliable on tolerance Days 3, 4, and 5 and on reverse Day 1 ($p<0.05$ by t-test).

Results and Discussion

Figure 2 presents the data for the final portion of the experiment: six days on which both groups received marihuana after testing, seven days over which tolerance was developed to 32 mg THC/kg, and one reversal day. The initial application of 8 mg THC/kg before testing did not suppress responding. The initial applications of 16, 24, and 32 did suppress responding (t=3.32, df=18, $p<0.01$; t=4.25, df=18, $p<0.001$; and t=4.32, df=18, $p<0.001$ respectively). Complete behavioral tolerance to 32 mg THC/kg was reached on the seventh day. During this period the baseline of responsing in the THC-after animals declined somewhat, though less than the similar decline in the first study. On the reversal day, the group shifted from THC-after to THC-before showed little evidence of tolerance. This group responded far less than the group shifted from THC-before to THC-after (t=4.09, df=18, $p<0.001$). Thus even with very high doses, learning appears to account for a significant proportion of the observed behavioral tolerance.

GENERAL DISCUSSION

An experiment with lever pressing for food reinforcement and employing intraperitoneal marihuana administration, and a second experiment with lever pressing for water reinforcement and employing oral marihuana administration have demonstrated that the experience of responding while drugged is a significant contributor to the development of behavioral tolerance to marihuana. Animals that received the drug following testing each day showed little evidence of tolerance when given the drug before testing. Thus the experiments provide evidence that a learning mechanism is involved in the development of behavioral tolerance to marihuana.

There are several possible ways in which learning might play a role in the development of tolerance. The most completely elaborated hypothesis is that behavioral tolerance to marihuana is a form of state dependent learning. The hypothesis proposes that the central state induced by marihuana places the animal in a stimulus situation that is very different from the undrugged condition. When pretraining is carried out in an undrugged condition, the animal learns to perform the response under one set of stimuli. The introduction of marihuana alters this set of stimuli so much that the animal is no longer able to perform well. Animals given marihuana before testing, however, have the opportunity to relearn the response under the new conditions. Animals given the drug after testing have no opportunity to relearn the response under the drug produced stimulus conditions, and thus fail to demonstrate tolerance when the drug is finally given before the test.

An alternative to the state dependent learning hypothesis is the idea that the animal may learn new responses to cope with or compensate for the effects of the drug. It is impossible to specify at this time, however, exactly what responses might be involved.

The observation that the development of behavioral tolerance to marihuana involves a learning process has implications for future investigations: (1) It may prove difficult or impossible to discover chronic, widespread biochemical changes such as alterations in the level or turnover of neural transmitter substances, which correlate with the development of behavioral tolerance. If behavioral tolerance involves a learning process, such changes would be expected to be restricted to distinct populations of neurons and would be impossible to measure by assaying entire regions of the brain. (2) Studies which do attempt to reveal

physiological or biochemical changes which underlie the development of behavioral tolerance must be carried out on animals which have actually demonstrated behavioral tolerance. Mere application of marihuana to the rat is, according to the present data, insufficient to develop behavioral tolerance. Thus, the animal that receives drug injections without behavioral testing cannot serve as a model for the biochemical investigation of behavioral tolerance.

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