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Residual Effects of Prolonged Cannabis Administration on Exploration and DRL Performance in Rats

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Abstract. Chronic oral administration of cannabis extract to rats (daily Δ^9-tetrahydrocannabinol dose 20 mg/kg) was examined for its residual effect on open field activity and DRL (differential reinforcement of low-rate responding) performance, following a 2–3-month drug-free period. Locomotor activity during the latter part of an open field test was markedly increased in rats previously treated for either 6 months or 3 months with the drug. The same treatments also produced a significant impairment on a DRL-20 task relative to control subjects' performance. These and other findings (impaired maze learning and facilitated two-way shuttle box avoidance) might mean that cannabis produces long-lasting hippocampal dysfunction in rats.

Key words: Chronic cannabis – Locomotor activity – DRL performance – Rat

Previous work from our laboratory has demonstrated retardation of learning of the Hebb-Williams maze (Fehr et al. 1976) or radial-arm maze (Stiglick and Kalant 1982) in rats which are examined at least 1 month after the end of a 3–6-month period of daily treatment with cannabis extract, with an oral dose providing 20 mg/kg Δ^9-tetrahydrocannabinol (THC). Since adequate performance on these tests has been shown to depend upon the integrity of the hippocampus (Kimble 1978; Olton et al. 1977; Olton and Werz 1978), it is possible that long-term cannabis administration produces a variety of behavioral changes which are similar to those caused by hippocampal lesions. The purpose of the present study was to examine this idea further, by using an open field test and an operant task involving a schedule of differential reinforcement of low-rate responding (DRL). Animals with hippocampal lesions exhibit more activity than controls in the open field (Kimble 1975; Myhrer 1975) and impaired DRL performance (Clark and Isaacson 1965; Rickert et al. 1973; Johnson et al. 1977).

Materials and Methods

The subjects were 73 male Wistar rats that had been tested on an eight-arm radial maze in previous experiments: For a detailed account of the subjects, cannabis extract, and intubation procedure see the companion paper (Stiglick and Kalant 1982). Twenty-nine of the animals had received either cannabis extract ($N = 15$) or vehicle substance ($N = 14$) for 6 months (6-month experiment) before being tested on the radial-arm maze. The remaining 44 subjects had received either cannabis ($N = 16$), vehicle ($N = 14$), or sham intubation ($N = 14$) for 3 months (3-month experiment) before testing on the maze began.

Open Field. The apparatus consisted of a roughly circular arena marked off into 19 equal hexagons, each with 10 cm sides. A single center hexagon was surrounded by six inner hexagons, which in turn, were surrounded by 12 outer hexagons along the perimeter of the arena. The arena was enclosed by a wall 36 cm high and by a conical hood 88 cm in diameter and 34 cm high. The hood was positioned 51 cm above the floor, and contained four light bulbs to illuminate the arena evenly. A layer of muslin across the bottom of the hood acted as a diffusing screen, providing an intensity of 165 foot-candles at floor level. A screen made of muslin covered the 15 cm gap between the hood and the top of the wall. A sliding door (11 × 10 cm) was located on one side of the apparatus to permit entry of the subjects.

DRL Apparatus. DRL tests were conducted in eight standard operant chambers (31.5 × 30.5 × 35.0 cm). Along one wall of each chamber was located a lever 6 cm above the grid floor, with a food cup to the right of the lever. Single 45 mg Noyes pellets were used as reinforcement.

Testing Procedure for 6-Month Experiment. A single open field test was conducted on rats 66 days after the last drug or vehicle treatment (post-drug period). Animals were placed individually into the arena for exactly 5 min. For seven of the subjects in each group, a single Noyes pellet (food condition) was placed within the center hexagon. The number of center, inner, and outer hexagons entered by the head and forepaws was counted for each minute. Data were analyzed by a four-way analysis of variance (group- × -food- × -hexagon type- × -time) with repeated measures on the last factor (Winer 1971). In order to interpret the significant interactions, Dunnett's a posteriori comparisons (Dunnett 1955) were used to compare the treatment groups at the beginning and at the end of the test. Intergroup comparisons were made with respect to the total activity during the first 2 min of the test, and also during the final 2 min of the test.

Bar-pressing tests commenced at 91 days postdrug. On day 1, subjects were magazine-trained for 20 min: Food pellets were delivered randomly at an average rate of 3/min to each operant chamber, with each lever inactivated. The rats

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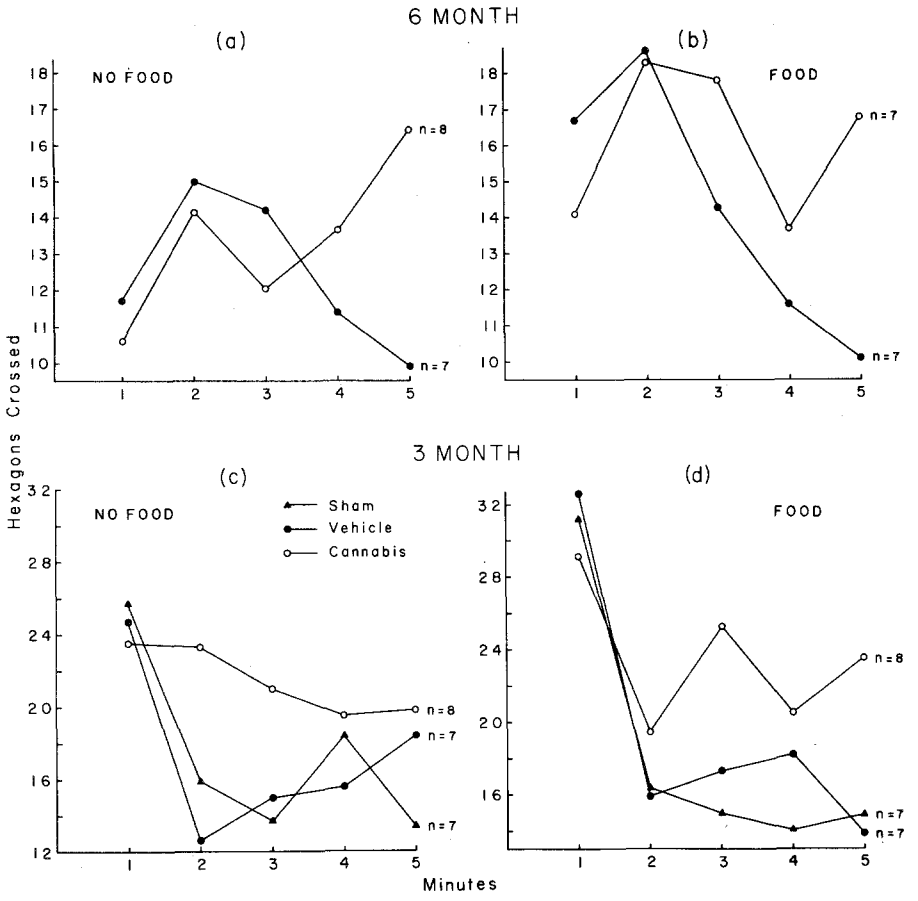

Fig. 1 a—d. Open field activity of cannabis-treated and control rats. Cannabis animals had received daily gavage during 6 months (**a, b**) or 3 months (**c, d**) with a dose of cannabis extract providing THC 20 mg/kg in olive oil. In the 6- and 3-month experiments testing occurred 66 and 79 days after the last gavage respectively. Panels **a** and **c** show activity of rats without any food in the arena. Panels **b** and **d** show activity of rats tested with a single Noyes pellet in the center hexagon

were immediately placed on a continuous reinforcement (CRF) schedule for 15 min, during which each bar press was reinforced with one food pellet. No attempt was made to shape the animals by the experimenter. CRF training was conducted for 30 min on each of the following 2 days and for 20 min on days 4–6. Following this the subjects were run for 16 test days on a DRL-20 schedule, under which reinforcement was received only for bar presses which followed a delay of at least 20 s after the previous response. All DRL sessions were 30 min in duration, and a light beside each chamber signalled the onset (light on) and termination (light off) of each session. A PDP-11 computer (Digital Equipment Corporation, Ottawa, Canada) controlled the schedule of reinforcement in the operant chambers, and recorded the number of bar presses and reinforcements on a teletype. A daily efficiency score [(number of pellets/number of responses) × 100] was calculated for each subject to assess its learning of the DRL-20 task. All testing was completed 112 days after the last intubation. Data were analyzed by two-way analyses of variance (group- × -days) with repeated measures on the last factor, and also by multiple regression analysis of the slopes of the learning curves.

Testing Procedure for 3-Month Experiment. A single open field test was conducted on rats at 79 days postdrug by the procedure already described. For half the subjects in each group a single Noyes pellet was placed in the center hexagon. On day 95 postdrug, open field tests were repeated for the vehicle- and cannabis-treated animals for 7 min each, with a single Noyes pellet placed in the center hexagon for every subject.

DRL-20 tests began 113 days after the last intubation. Both the CRF training and DRL testing were conducted as already outlined. Subjects were run for 24 test days on the DRL schedule.

Data for both the open field tests and DRL-20 sessions were analyzed by the methods used in the 6-month experiment.

Results

Open Field. As shown in Fig. 1 a, b, animals in the 6-month experiment which received a single Noyes pellet in the open field apparatus were slightly more active at the beginning of the session than rats which were given no pellet, but this effect was not statistically significant. Irrespective of the presence of food, there was a clear negative slope of the activity versus time curve for vehicle controls, but not for cannabis-treated subjects (significant group- × -time interaction $F = 2.58$, df 4,100, $P < 0.04$). This difference was due to the fact that activity was not significantly different between the groups during the first 2 min, whereas the activity of cannabis-treated animals was significantly higher during the last 2 min ($t = 2.11$, df 27, $P < 0.04$). There was also a definite trend for

cannabis-treated rats to enter more of the outer hexagons than vehicle controls during the latter part of the test, but this trend did not quite reach the 5% level of significance (group- × -hexagon type- × -time interaction $F = 1.87$, df 8, 200, $P < 0.06$).

Figure 1c, d also shows the results of the first open field test conducted on animals in the 3-month experiment. Analysis of variance indicated that, in both treatment groups, the presence of a food pellet in the arena increased exploration, especially at the beginning of the test (significant food- × -time interaction $F = 2.95$, df 4,152, $P < 0.02$; group- × -food and group- × -food- × -time interactions were not significant). Regardless of the presence of food, the slopes of the activity curves were significantly different among the three groups of subjects (significant group- × -time interaction $F = 3.19$, df 8,152, $P < 0.002$). A posteriori comparisons showed that activity among the groups was not significantly different during the first 2 min of the test, whereas the cannabis-treated animals were much more active than both the vehicle ($t = 2.32$, df 28, $P < 0.05$) and sham ($t = 3.12$, df 28, $P < 0.01$) controls during the last 2 min. There was no significant difference between the two control groups during the latter part of the test. The greater activity of cannabis-treated subjects was due mainly to a higher number of outer hexagons crossed in the latter portion of the test (significant group- × hexagon type- × -time interaction $F = 3.03$, df 16,304, $P < 0.001$).

Essentially similar results were obtained when cannabis-treated and vehicle-treated subjects were retested 16 days later in the open field. Compared with controls, rats in the cannabis group exhibited a marked hyperactivity during the latter part of the test, due to a much higher number of outer hexagons crossed (significant group- × -hexagon type- × -time interaction $F = 1.96$, df 12,336, $P < 0.03$).

The pattern of activity exhibited by cannabis-treated rats in all open field tests was also different from that of control animals. Throughout the tests the cannabis-treated rats tended to run rapidly along the perimeter of the arena, stopping only rarely, while behavior of control subjects consisted primarily of "bursts" and "stops", with more rearing and grooming between bursts of activity.

DRL-20. In the 6-month experiment, all animals readily learned to bar press under the CRF schedule, and no significant differences were found between the cannabis and vehicle groups over the 6 days of CRF training. However, as shown in Fig. 2a, under the DRL-20 schedule cannabis-treated subjects learned at a much slower rate than vehicle controls (significant group- × days interaction $F = 4.46$, df 15,390, $P > 0.007$), even though the actual number of bar presses made was not significantly different between the groups. The difference in rate of learning was confirmed by multiple regression analysis (significant slope difference $F = 16.57$, df 1,444, $P < 0.001$). The lower efficiency scores were due to the fact that rats in the cannabis group tended to wait less than 20 s after each bar press and, thus, acquired fewer food pellets. Time distributions of bar pressing, for representative rats of the two groups, are shown in Fig. 3a.

Very similar results were obtained from subjects in the 3-month experiment: No significant differences were observed between the cannabis and vehicle groups on CRF training. In contrast, Fig. 2b shows that cannabis-treated animals performed more poorly on the subsequent DRL tests than control animals (significant group- × -days interaction

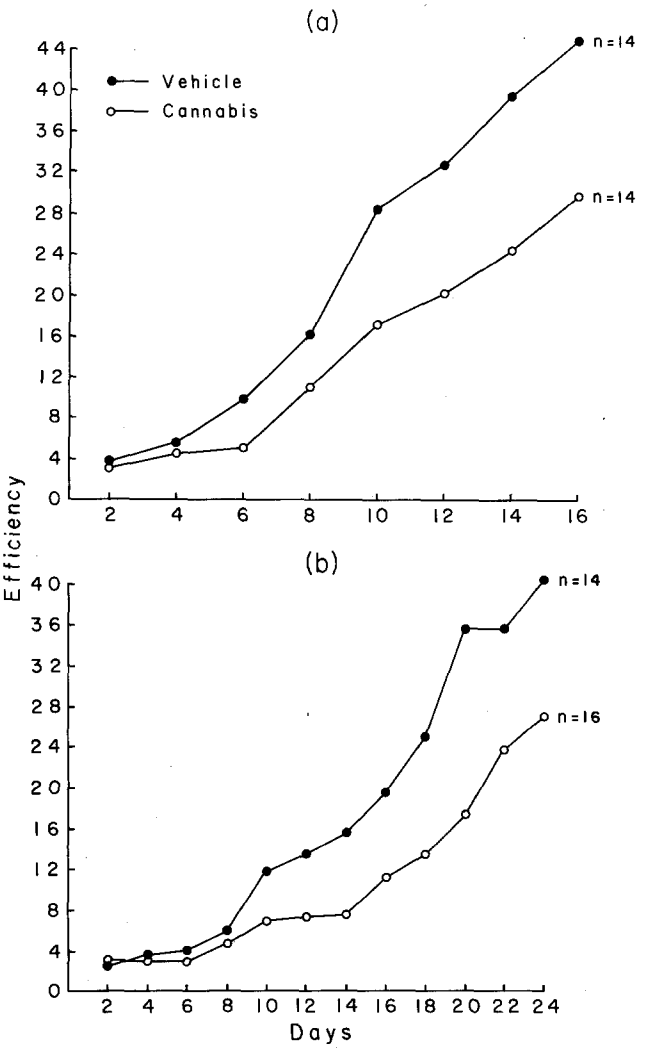

Fig. 2a, b. Acquisition of DRL-20 performance by cannabis-treated and control rats following either 6 months **a** or 3 months **b** of intubation (THC dose 20 mg/kg). In the 6- and 3-month experiments testing began 97 and 113 days after the last gavage respectively. Percent of responses reinforced (efficiency score) is calculated as [(number of pellets obtained/number of bar presses) × 100]

$F = 1.79$, df 23, 644, $P < 0.01$), while the actual number of bar presses was not significantly different. The difference in learning rates was confirmed by multiple regression analysis (significant slope difference $F = 17.06$, df 1, 716, $P < 0.001$). The lower efficiency scores in the cannabis group were again due to failure to wait at least 20 s between bar presses, resulting in a lower number of food reinforcements (Fig. 3b).

Discussion

The present results demonstrate that hyperactivity in the open field and impaired learning of a DRL-20 task can be produced by as little as 3 months of chronic cannabis administration, if the cannabis extract is adjusted to provide a daily dose of 20 mg THC/kg. These data extend our previous findings that the same daily dose, given for 3–6 months, impaired the learning of the Hebb-Williams maze (Fehr et al. 1976) and the radial-arm maze (Stiglick and Kalant 1982).

Because the learning impairment on the DRL-20 task was evident up to 136 days after the last drug treatment, it is

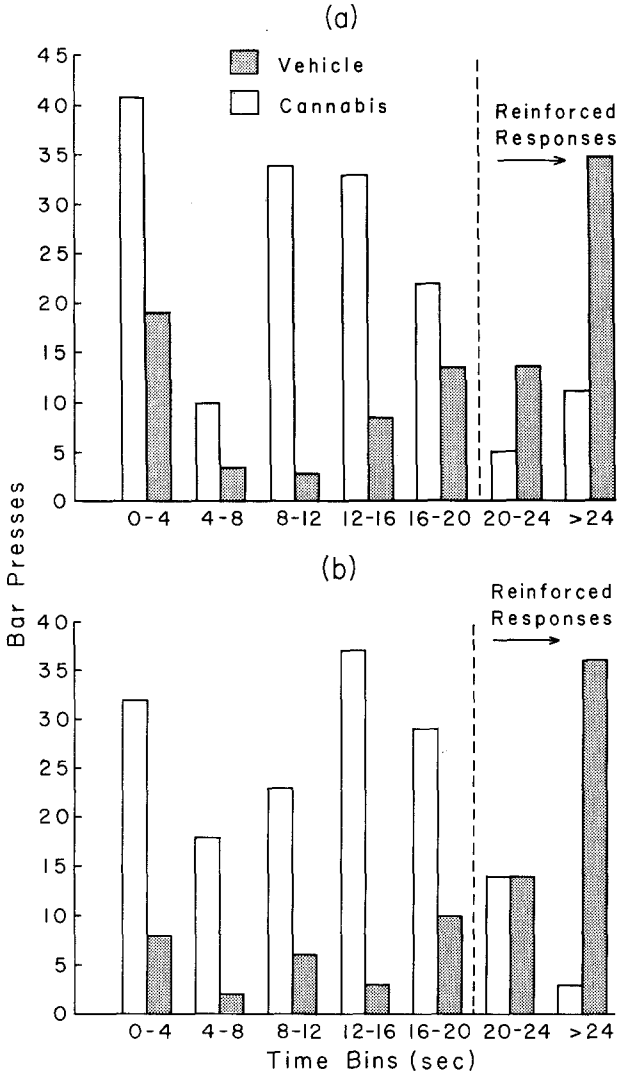

Fig. 3a, b. Cumulative frequency of responding at various times after a previous bar press for a typical cannabis-treated subject (*open bars*) and vehicle-treated subject (*solid bars*) from the 6-month **a** and 3-month **b** experiments. Scores were obtained from the final day of testing. Responses were reinforced only if at least 20 s had elapsed since the previous bar press

extremely unlikely that the behavioral changes were due to residual drug in cannabis-treated subjects. Furthermore, since the sham and vehicle controls did not differ from each other in the open field, nor on acquisition of radial-arm maze performance in a previous study (Stiglick and Kalant 1982), it is unlikely that the differences between rats in the cannabis and vehicle groups were due to an ameliorative effect of the vehicle substance (olive oil).

In view of the fact that such findings are clearly attributable to prolonged cannabis administration, it is important to explain the mechanisms by which cannabis produces long-term behavioral changes. One possibility is that animals exposed to prolonged cannabis treatment are generally more active in the testing environments used. This might account for the hyperactivity in the open field exhibited by cannabis-treated rats. However, this idea cannot explain all of the data because, on the DRL task, there was no significant difference in the actual number of bar presses made by cannabis-treated subjects and vehicle controls. Moreover, this explanation does not account for the fact that the same cannabis-treated animals exhibited a lower rate of entry into the arms of the eight-arm radial maze (Stiglick and Kalant 1982).

A second possibility is that long-term cannabis administration produces a residual fear of open spaces. This might explain the fact that most of the increased activity of cannabis-treated rats in the open field occurred near the perimeter of the arena, in the outer hexagons. This is consistent with the finding that rats in the cannabis groups were reluctant to leave the center platform of an eight-arm radial maze on test day 1. However, it does not explain the learning impairment on a 12-arm radial maze in which there was no evidence that cannabis-treated animals were more reluctant to leave the center platform than vehicle controls (Stiglick and Kalant 1982). Nor does such a change in vigilance explain the learning deficits on the DRL-20 task.

A third possibility is that one of the residual effects of long-term cannabis treatment is to alter the rat's ability to inhibit responding. In the open field, normal rats quickly learn to inhibit their natural tendency to explore after a few minutes, while cannabis-treated animals remain active in the outer portions of the arena, in spite of waning novelty. Similarly, on the DRL task, control rats learn to inhibit the continuous bar pressing behavior that was reinforced on a CRF schedule, whereas rats in the cannabis groups are less efficient on DRL tests because they respond as though reinforcement were still being delivered after each bar press. This type of deficit may have also contributed to the learning impairment on the eight-arm radial maze, in which cannabis-treated animals tended to exhibit a higher proportion of "perseverative" errors than control subjects.

Since it is well known that hippocampal lesions often affect the rat's ability to inhibit responding (Kimble 1975; Black et al. 1977), it is interesting to note the general similarity between the effects of long-term cannabis administration and hippocampal lesions: Both treatments produce slower learning of the Hebb-Williams maze (Fehr et al. 1976; Kimble 1978) and radial-arm maze (Olton et al. 1977; Olton and Werz 1978), hyperactivity in open fields (Kimble 1975, 1978; Myhrer 1975), and impaired performance on DRL tests (Clark and Isaacson 1965; Rickert et al. 1973; Johnson et al. 1977). Moreover, chronic cannabis administration accelerates acquisition of shuttle box avoidance (Stiglick and Kalant, data in preparation) and this effect also accompanies hippocampal lesions (Isaacson et al. 1961; Olton and Isaacson 1968). Although there are many alternative explanations which may account for this similarity, it is possible that long-term cannabis treatment results in hippocampal dysfunction. However, the residual effects of cannabis administration do not prove the existence of identifiable histological lesions in drug-treated animals. This is the subject of a separate investigation now in progress.

The possibility that chronic heavy cannabis use might produce such residual learning deficits in humans cannot be ruled out a priori on the grounds that the dosage in these experiments is far in excess of that used even by heavy smokers of cannabis. If dosage is expressed per square meter of body surface rather than per kilogram of body weight, to correct for differences in metabolic rate between large and small animals (Rosenkrantz and Braude 1976), and if allowance is made for the fact that intensity of cannabis effects is only about one-third as great after oral administration as after smoke inhalation (Kiplinger and Manno 1971), then the dosage used in these experiments is well within the limits used

by heavy smokers. Rosenkrantz and Fleischman (1979) have recently reported that the use of such dosage corrections for species and route yields virtually identical blood levels of THC in rats and humans. As noted previously, the cannabis-treated rats in the present experiments were not grossly intoxicated, and after 3–4 weeks of treatment they could not be distinguished behaviorally from the controls (Stiglick and Kalant 1982). Whether residual learning deficits do occur in human users of cannabis can be settled only by suitably large-scale observations (preferably prospective studies) in matched groups of heavy users and nonusers.

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