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Residual effects of chronic cannabis treatment on behavior in mature rats

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Abstract. Mature rats (starting weight at least 270 g) were treated daily with cannabis extract (daily THC dose 20 mg/kg) for 3 months. After a 1- to 4-month drug-free period, residual effects on a variety of behaviors were studied. No residual effects were found in learning of an eight-arm radial maze task, nor on a differential reinforcement of low-rate responding (DRL-20) task, nor on open field activity. On the other hand, two-way shuttle box avoidance learning was facilitated by previous cannabis treatment, since cannabis-treated rats exhibited shorter mean latencies to avoid footshock than vehicle controls. The findings indicate greater vulnerability of immature organisms (previous studies) than mature organisms (the present study) to long-term effects of chronic cannabis administration.

Key words: Mature rats – Chronic cannabis – Learning – Activity – Radial maze – Open field – DRL-20 operant behavior – Shuttle box

Recent studies from our laboratory have revealed residual effects of chronic cannabis treatment on behavior in the laboratory rat. In these studies rats were intubated daily for 3–6 months with cannabis extract, in a dose providing 20 mg of Δ^9-tetrahydrocannabinol (THC)/kg. Residual effects were observed at least 1 month after the last drug treatment. These effects consisted of retarded learning in a Hebb-Williams maze (Fehr et al. 1976), a radial-arm maze (Stiglick and Kalant 1982a), and a DRL-20 bar pressing task (Stiglick and Kalant 1982b). There was also residual hyperactivity in an open field test (Stiglick and Kalant 1982b) and facilitation of two-way shuttle box avoidance (Stiglick et al. 1984). Although these residual effects were observed in adult rats weighing more than 300 g, drug treatment had begun when the subjects were immature, since they weighed only 120–130 g (age about 40 days) during the first intubation.

The purpose of the present study was to determine the residual effects of the same chronic cannabis treatment in rats that were mature before drug treatment. Animals that weighed at least 270 g (age about 70 days) were intubated daily with cannabis extract for 3 months, using the same daily dose of THC (20 mg/kg) as in previous studies. Following a 1 month recovery period, the subjects were exposed to a battery of the most sensitive behavioral tests used in previous studies: the radial-arm maze, open field, DRL-20, and shuttle box avoidance tests.

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Materials and methods

The subjects were 32 male Wistar rats weighing 270–275 g at the beginning of drug treatment. They were housed individually, exposed to a 12-h light-dark cycle (lights on 7 AM) and given free access to both food and water until they had attained a weight of 320–330 g. They were maintained at this weight by food restriction as described by Stiglick and Kalant (1982a). The rats were fed at the end of the day, following treatment or behavioral testing, to ensure that they were adequately motivated for tests involving food reward.

Cannabis extract. Dried cannabis leaf material (marijuana) was obtained from the Department of National Health and Welfare, Ottawa. Preparation and analysis of the cannabis extract have been described previously (Stiglick and Kalant 1982a). The extract contained 22.1% THC, 3.6% cannabinol, and 0.9% cannabidiol, expressed as dry weight. For intubation the extract was dissolved in olive oil to give a concentration of 10 mg of THC/ml solution, and the same supply of olive oil served as the control substance for vehicle-treated animals. New solutions were prepared from stock every 1–2 weeks.

Intubation procedure. The rats were randomly assigned to either the cannabis group (n = 16), which received daily gavage at a dose of 20 mg THC/kg body weight, or the vehicle group (n = 16), which was given an equivalent volume of olive oil. All subjects received their respective treatments for 90 days, followed by a drug-free period of 31 days without tests. Subsequent behavioral tests were then conducted over the next 94 days using a testing schedule very similar to that used in previous experiments (e.g., Stiglick and Kalant 1983).

Radial-arm maze tests. The apparatus, procedure, dependent measures, and statistical treatment were very similar to those used previously (Stiglick and Kalant 1982a, experiments 1 and 2). The apparatus consisted of eight radiating arms attached at equal distances from each other to a center platform that was 50 cm in diameter. Each arm was 85 cm long and 10 cm wide, equipped with a small light and photocell used to indicate arm entry by the rat (see Stiglick and Kalant 1982a, experiment 3, for further details

of arm entry). Each arm contained a small well at the end that served as a food cup for a 45-mg Noyes pellet.

Testing was carried out 7 days/week between days 31 and 55 after the last intubation (postdrug period). On each test day a rat was placed individually at the middle of the center platform. Each test was terminated after 10 min, or when the animal had obtained all eight food pellets, whichever occurred earlier. Each rat was tested on alternate days for 10 sessions, then on consecutive days for five more sessions (total of 15 sessions).

The mean numbers of "correct" and "error" scores on each test day were used as measures of learning ability. Correct responses were defined as the number of arms entered only once in the first eight entries made. Errors were defined by the number of arms that a rat entered more than once. In addition, a criterion of "perfect" performance was used as an overall measure of learning ability. An animal reached this criterion on the 1st day it achieved a correct score of 8/8.

Open field tests. The apparatus consisted of a roughly circular arena marked off into 19 equal hexagons (see Stiglick and Kalant 1982b for details of apparatus and procedures). A single Noyes pellet was placed in the center hexagon. For each test a rat was placed individually into the arena for exactly 7 min. The number of center, inner and outer hexagons entered by the head and forepaws was counted for each minute from videotaped records. Each subject was tested twice in the open field at 59–60 days postdrug.

DRL-20 tests. DRL tests were conducted in standard operant chambers (see Stiglick and Kalant 1982b for details of apparatus and procedures). Barpressing tests were carried out 87–114 days postdrug. After 1 day of "magazine" training (day 1) and a continuous reinforcement (CRF) schedule (days 1–6), the subjects were run 7 days/week for 22 sessions on a DRL-20 schedule. Reinforcement was received only for the first bar press that followed a delay of at least 20 s after the previous response. A daily efficiency score [(number of pellets/number of responses) × 100] was calculated for each subject.

Shuttle box avoidance tests. The apparatus consisted of a two-way shuttle box with a metal barrier separating the two compartments (see Stiglick and Kalant 1983 for more details). The conditioned stimulus (CS) consisted of a light and a tone presented together. The light stimulus was the onset of a cue light on the side of the chamber occupied by the subject at the beginning of each trial. The unconditioned stimulus (UCS) was a 0.6-mA shock, and the CS-UCS interval was 7 s. Both the CS and UCS stayed on until the animal avoided or escaped shock by going to the other ("safe") side of the apparatus, and then terminated simultaneously. An "avoidance response" occurred whenever the subject moved to the safe side of the chamber during the CS-UCS interval, i.e., before the shock was delivered.

Avoidance training was carried out 116–125 days postdrug. The subjects were tested 7 days/week until 10 days of data were collected. Each of the 10 avoidance sessions consisted of 20 trials with an intertrial interval of 30 s.

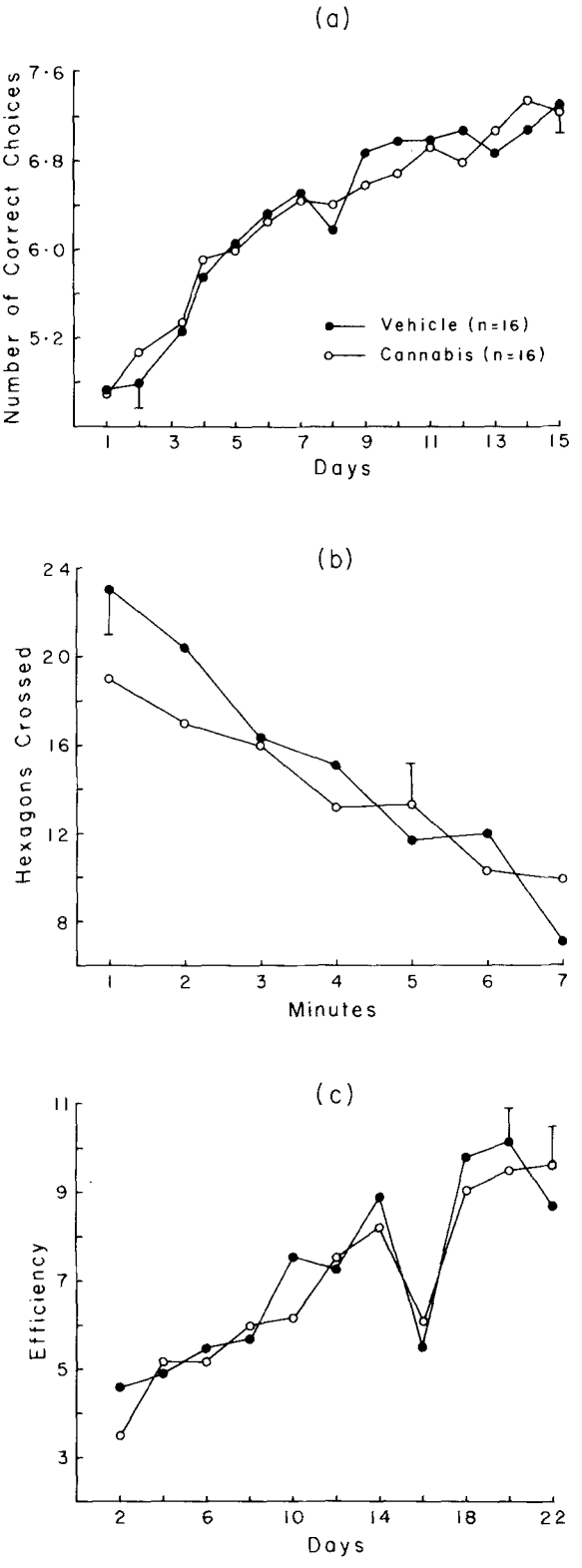

Fig. 1a–c. Maze learning (**a**), open field activity (**b**) and DRL-20 learning (**c**) in previously cannabis-treated and control rats. Cannabis animals had received daily gavage during 3 months with a dose of cannabis extract providing THC 20 mg/kg in olive oil; controls had received only olive oil. **a** Correct performance scores on the maze. The largest SEM for each group is shown. Testing occurred between 31–55 days postdrug. **b** Hexagons crossed in the first open field test at 59 days postdrug. **c** Acquisition of DRL-20 performance between 93–114 days postdrug. Percent of responses reinforced (efficiency score) is calculated as [(number of pellets obtained/number of bar presses) × 100]

Results

Analyses of variance revealed no significant differences between the cannabis and vehicle groups on any measure, except the shuttle box avoidance tests. For example, the ability of cannabis-treated animals to learn the radial-arm maze was equivalent to that of vehicle-treated rats, as measured by correct scores (Fig. la); there were also no differences in error scores or in the perfect performance criterion on the maze. Similarly, there was no evidence for differences in activity in the open field tests (Fig. 1b shows the results for the first open field test; virtually identical results were obtained for the second open field test), nor for differences in learning ability on the DRL tests (Fig. 1c).

Figure 2 shows the residual effects of chronic cannabis treatment on two-way shuttle box avoidance. Although there was a trend for cannabis-treated rats to exhibit more avoidance responses than vehicle controls (Fig. 2a), this effect was not statistically significant ($P > 0.1$). On the other hand, rats in the cannabis group did have significantly shorter mean latencies to avoid the footshock (Fig. 2b) during most sessions (main effect of group: $F = 6.92$, df 1,29, $P < 0.01$; group by session interaction was not significant). There was also a trend for cannabis-treated animals to exhibit more spontaneous crossings during the intertrial interval, but this effect was not significant.

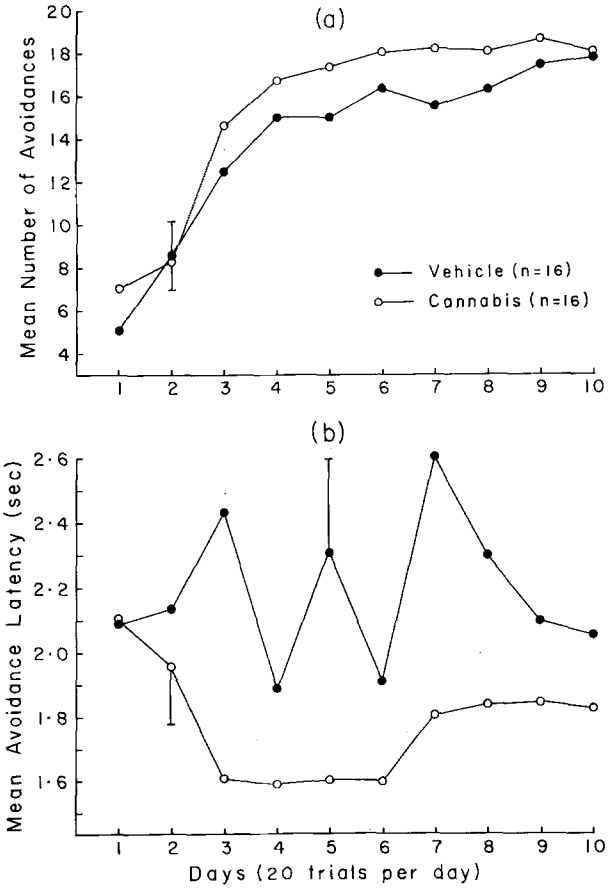

Fig. 2a, b. Learning of shuttle box avoidance by previously cannabis-treated and control rats. Testing occurred between 116 and 125 days postdrug. **a** Mean number of avoidances on successive test days. The largest SEM for each group is shown. **b** Mean avoidance latencies on successive tests

Discussion

This experiment demonstrates that 3 months of chronic cannabis treatment in mature rats has very few residual effects on behavior, if the animals weight at least 270 g when drug treatment begins. This is in sharp contrast to the residual effects of the same drug treatment in immature animals weighing only 120–130 g at the start of drug exposure. In the latter case there was residual learning impairment on the radial-arm maze (Stiglick and Kalant 1982a) and the DRL-20 task (Stiglick and Kalant 1982b), and residual hyperactivity in the open field (Stiglick and Kalant 1982b). The previous studies in immature rats and the present experiment differ only in the starting weight and age of the animals; all other procedures, including dose of drug, duration of drug treatment, and behavioral testing schedules are virtually identical. This suggests a greater vulnerability of immature rats to the residual effects of chronic cannabis adminstration.

The only significant effect in the present study was on shuttle box learning, since cannabis-treated rats exhibited shorter mean latencies to avoid the footshock than vehicle controls did. This effect on avoidance latency has been shown previously in two experiments on immature rats, which also displayed significantly more avoidance responses than controls on this test (Stiglick et al. 1984). Therefore, the present shuttle box findings are consistent with previous observations, but are less marked. This again suggests that immature rats are more vulnerable than mature animals to the residual effects of the drug.

The daily dose of cannabis used in the present study was the same as in our previous experiments (20 mg THC/kg), and was not unusually high. Rosenkrantz (1983) has reported that PO doses of THC ranging from 6 to 30 mg/kg/day in the rat are directly relevant to human consumption, and may be considered as "moderate" when compared with human use of cannabis.

In an earlier paper we speculated that the residual *facilitation* of shuttle box avoidance in immature, cannabis-treated rats might be due to hippocampal dysfunction (Stiglick et al. 1984). The present resutls suggest that this or some other change in the brain is less marked in mature animals. Presumably, the immature rat brain is more vulnerable than the mature brain, because the former is not fully developed until the rat is about 2 months old (Douglas 1975). Thus, in our earlier studies, cannabis treatment was started at an age when functional connections between neurons may still have been forming, and drug action might have prevented this process from being completed. Future studies should address this possibility directly, by histological, electrophysiological, or neurochemical examination of the brains of immature and mature cannabis-treated animals.

It is obvious that caution must be exercised in any attempt to extrapolate results from animal experimentation to man. In spite of this constraint, however, the present results, along with previous studies on immature rats, suggest that immature organisms may be more susceptible to chronic cannabis treatment than mature organisms. Whether residual effects do occur in human users of cannabis, and whether these effects are more marked in immature users, can be settled only by suitably large-scale observations (preferably prospective studies) in matched

groups of immature and mature heavy users and immature and mature non-users.

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