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Residual effects of prolonged cannabis treatment on shuttle-box avoidance in the rat

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Abstract. Chronic oral administration of cannabis extract to rats was examined for its residual effects on shuttle-box avoidance learning. In experiment 1 avoidance learning was assessed in rats that had been tested previously on other behavioral tests. Chronic treatment (3 months) facilitated the learning of shuttle-box avoidance in cannabis-treated animals relative to vehicle controls. In experiment 2 very similar results were obtained in naive rats. These and other residual effects of chronic cannabis treatment are similar to the effects of hippocampal lesions.

Key words: Chronic cannabis – Shuttle-box avoidance – Rat

Previous studies in our laboratory have shown that prolonged administration of *Cannabis sativa* to rats can produce residual effects on many 'appetitive' tasks involving food reward. For example, rats that are intubated daily for 3–6 months with cannabis extract, in a dose providing 20 mg/kg Δ^9-tetrahydrocannabinol (THC), show retarded learning on a Hebb-Williams maze (Fehr et al. 1976) and a radial-arm maze (Stiglick and Kalant 1982a), and slower acquisition on a DRL-20 bar-pressing task (Stiglick and Kalant 1982b). These effects on appetitive learning are observed 1–6 months after the last drug administration.

The purpose of the present study was to determine the residual effects of prolonged cannabis treatment on avoidance learning using a two-way shuttle-box test. A previous experiment by Radouco-Thomas et al. (1976) suggested that long-term treatment with THC facilitated two-way active avoidance learning in mice. The present work reports a similar facilitation in rats following prolonged cannabis treatment. This was demonstrated in two separate experiments. In the first, the rats had undergone previous testing in other behavioral paradigms before the avoidance learning. In the second, avoidance learning was studied in test-naive animals to rule out possible confounding effects of previous learning.

Materials and methods

Experiment 1. The subjects were the same animals that were used in work reported earlier (Stiglick and Kalant 1982a). They consisted of 28 male Wistar rats, weighing 120–130 g at the beginning of drug treatment (approximately 40 days of age). They were housed individually, exposed to a normal light-dark cycle, and given free access to both food and water until they had attained a weight of 320–330 g. Their weights were then maintained at this level by a daily ration of three to four standard rat chow pellets, and body weights were monitored at least twice per week.

Dried cannabis leaf material (marijuana) was obtained from the Department of National Health and Welfare, Ottawa, Canada. Preparation and analysis of the cannabis extract have been described previously (Stiglick and Kalant 1982a). The material contained 10.0% THC, 5.0% cannabinol (CBN) and 0% cannabidiol (CBD), expressed as dry weight of extract. For intubation the extract was dissolved in olive oil to give a concentration of 10 mg 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.

The animals were randomly assigned to either the cannabis group ($N = 14$) or the vehicle group ($N = 14$). Cannabis-treated rats received daily gavage of extract in a dose that provided 20 mg/kg THC, while rats in the vehicle group were given an equivalent volume of olive oil. All subjects received their respective treatments for 90 days (3 months).

The shuttle box apparatus consisted of a Lehigh Valley Electronics model 146-04 toggle-floor shuttle box (Fogelsville, PA, USA). Scrambled shock was delivered to either side of the chamber by a BRS Foringer model SGS-001 shock generator-scrambler (Beltsville, MD, USA). A tone was generated from a point directly over the center of the apparatus, either by a Mallory Sonalert (standard on the Lehigh Valley shuttle box), or by an Ashman Electronics model 64-SP tone generator (Greensville, Ontario, Canada), adjusted to an equivalent sound intensity at 2,000 cps. A cue light of 7.5 W was mounted on each end wall of the chamber.

At 30 days after the last drug or vehicle treatment (post-drug period) all rats were tested in a 12-arm radial maze and an open-field apparatus; the maze results have been reported previously (Stiglick and Kalant 1982a). Initial avoidance training on the shuttle box was begun 118 days post-drug and 38 days following the end of the previous tests. The subjects were tested 6 days a week until 10 days of data were collected. Each of the ten avoidance sessions consisted of 20 trials with an intertrial interval of 30 s. The conditioned stimulus (CS) was a compound

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stimulus of light and tone presented together. The light stimulus was the onset of the 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 7.0 s. Both the CS and UCS stayed on until the animal avoided or escaped shock by moving to the other ('safe') side of the apparatus, and then terminated simultaneously. An 'avoidance response' occurred whenever a subject moved to the safe side of the chamber during the CS-UCS interval, i.e., before the foot-shock was delivered. Starting at 194 days post-drug the animals were retested for an additional 4 days.

A PDP-11 computer (Digital Equipment Corporation, Ottawa, Canada) controlled the presentation of stimuli, and recorded the number of avoidance and intertrial interval responses and the mean latencies to avoid or escape during each session. The data were analysed by two-way analyses of variance (group-×-session) with repeated measures on the last factor (Winer 1971).

Experiment 2. The subjects were 26 naive male Wistar rats weighing 120–130 g (approximately 40 days of age) when drug treatment began. Animals in the cannabis ($N = 12$) and vehicle ($N = 14$) groups were housed and food-deprived as outlined in experiment 1.

The materials and procedure were identical to those of experiment 1, except for the following changes: (1) Different leaf material was used to make the cannabis extract, i.e., the material contained 22.1% THC, 3.6% CBN and 0.9% CBD, expressed as dry weight of extract; (2) shuttle-box testing began 30 days post-drug, with no previous exposure to any other tests; (3) the animals were only tested 6 days a week until 10 days of data were collected, i.e., there was no retest period.

Results

Experiment 1. As shown in Fig. 1, shuttle-box avoidance was facilitated in the cannabis group relative to vehicle controls. The mean number of avoidance responses made by cannabis-treated subjects was significantly higher (Fig. 1a), both in the initial 10-day test period (main effect of group $F = 5.89$, df 1,26, $P < 0.02$; group-×-session interaction NS) and in the 4-day retest period (main effect of group $F = 4.53$, df 1,26, $P < 0.04$; group-×-session interaction NS). In addition, subjects in the cannabis group exhibited a shorter mean latency to avoid the foot-shock (Fig. 1b) at the outset of the initial test period (significant group-×-session interaction $F = 2.73$, df 9,234, $P < 0.005$). Tests of simple effects (Winer 1971) showed

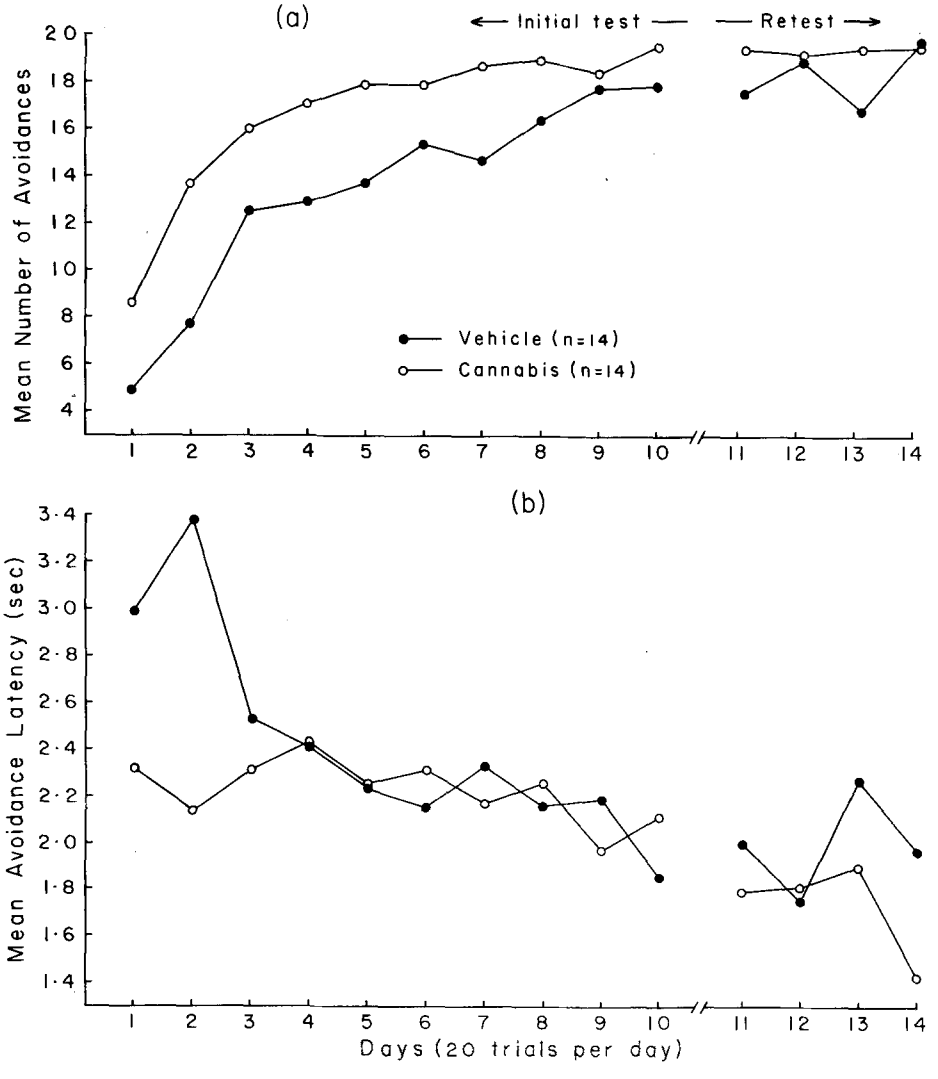

Fig. 1a, b. Learning of shuttle box avoidance by cannabis-treated and control rats in experiment 1. Cannabis animals had received daily gavage during 3 months with a dose of cannabis extract providing THC 20 mg/kg in olive oil; controls received only olive oil. Initial testing and retesting began 118 and 194 days after the last gavage, respectively: **a** Mean number of avoidances on successive test days; **b** mean avoidance latencies on successive tests

that this difference between the groups was significant on the first 2 days of testing (significant Student's t-test values 2.08 and 4.06 respectively; df 172, $P < 0.05$ in both cases). This shorter avoidance latency was also exhibited during the retest period, but was not statistically significant.

The faster learning of rats in the cannabis group was accompanied by a greater number of spontaneous crossings during the 30 s intertrial interval at the outset of the initial 10-day testing period (significant group-×-session interaction $F = 2.14$, df 9,234, $P < 0.03$). Tests of simple effects showed that this difference between the groups was confined to the first 4 days of testing (significant Student's t-test values 2.31–2.84; df 97, $P < 0.05$), during which the mean number of spontaneous crossings per interval was 1.23 ± 0.63 in the cannabis group and 0.61 ± 0.48 in the vehicle group. Because the number of spontaneous crossings declined in the cannabis-treated rats and increased in vehicle controls after the 4th testing day, this difference between the groups was not significant during the last 6 days of the initial test period. There was also no significant difference between the groups on this measure during the 4-day retest period; on the final session of that period the values for cannabis- and vehicle-treated subjects were 1.01 ± 0.73 and 1.00 ± 0.79 respectively.

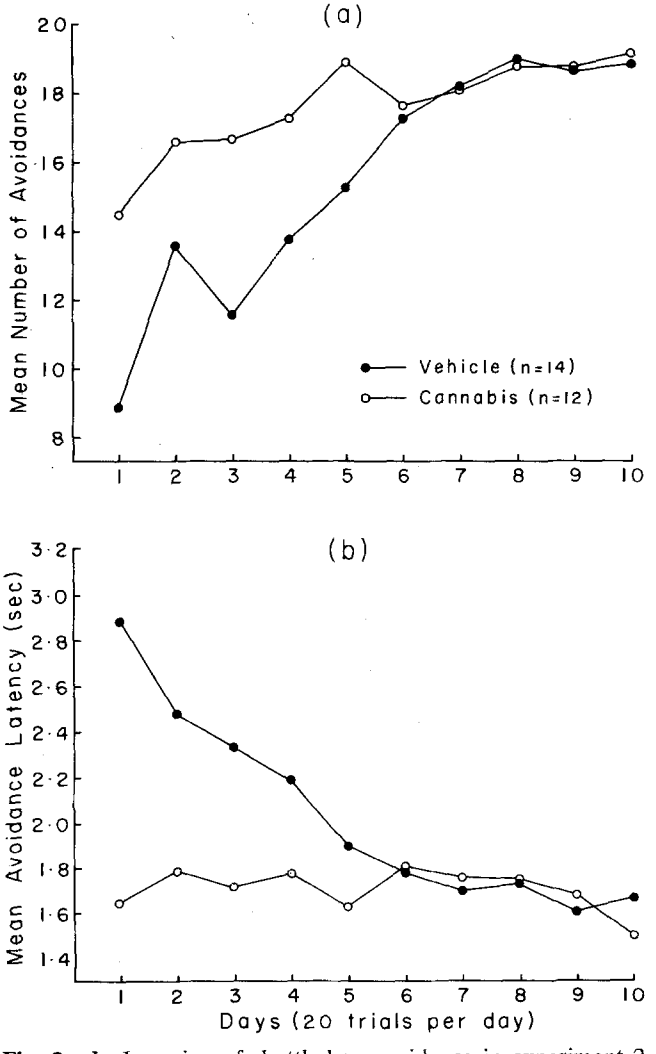

Fig. 2a, b. Learning of shuttle-box avoidance in experiment 2. Animals had received daily gavage for 3 months, as in experiment 1. Testing occurred 30–40 days after the last drug treatment: **a** Mean number of avoidances; **b** mean avoidance latencies

Experiment 2. Two-way avoidance was again facilitated in cannabis-treated rats relative to vehicle controls (Fig. 2). Initially the mean number of avoidance responses made by rats in the cannabis group was significantly higher (Fig. 2a; significant group-×-session interaction $F = 3.43$, df 9,216, $P < 0.0006$). Tests of simple effects showed that this difference between the groups was significant on the first 5 days of testing (significant Student's t-test values 2.11–4.29, df 181, $P < 0.05$ in all cases). In addition, cannabis-treated rats exhibited a shorter mean latency to avoid the foot-shock (Fig. 2b) at the outset of testing (significant group-×-session interaction $F = 1.90$, df 9,216, $P < 0.05$). Tests of simple effects revealed that this group difference was significant on the first 2 test days (significant Student's t-test values 3.79 and 2.14 respectively; df 217, $P < 0.05$ in both cases). There were no significant group differences in spontaneous crossings during the 30-s intertrial intervals.

Discussion

Experiment 1 demonstrates that acquisition of two-way active avoidance can be facilitated by 3 months of chronic cannabis administration, if the cannabis extract is adjusted to provide a daily dose of 20 mg/kg THC. Because the half-life of THC and its metabolites is approximately 21 h in the rat (Klausner and Dingell 1971), it is extremely unlikely that the effect on shuttle-box avoidance was due to residual drug in cannabis-treated animals 4–6 months after the last drug treatment. Instead it seems clear that the effect was due to a long-term, possibly permanent, change in the behavior of the drug-treated subjects.

Since experiment 1 was conducted on rats previously tested on other learning tasks (radial-arm maze and open field), it could be argued that the shuttle-box data were confounded by these previous tests. According to this argument, the superior learning of the control animals on the radial-arm maze (Stiglick and Kalant 1982a) may have interfered with their subsequent learning on the shuttle box (i.e., produced 'negative transfer'), leading to apparent facilitation in cannabis-treated rats.

Experiment 2 was conducted in naive animals. Therefore the facilitation of learning in the cannabis group could not have been due to confounding effects of previous testing. This suggests that the similar facilitation observed in experiment 1 was due only to the previous drug exposure.

The effect of cannabis seen here is consistent with the observations in mice (Radouco-Thomas et al. 1976): Chronic THC treatment resulted in a similar facilitation of shuttle-box learning in two of three strains of mouse tested, and the third strain did not differ from controls.

Although chronic cannabis treatment produced facilitation of active avoidance learning in the present studies, this effect cannot be generalized to all types of avoidance procedures. It is known that alterations of hippocampal function produce different effects on different avoidance behaviors. For example, low-intensity electrical stimulation of the hippocampus, following step-through passive avoidance conditioning in rats, produced opposite effects on the retention of conditioning under response-contingent versus

noncontingent shock (Kapp et al. 1978). Rats subjected to bilateral electrolytic lesions of the hippocampus made fewer crouching responses to electric foot-shock, and had more errors in passive avoidance learning (Blanchard and Fial 1968). It is therefore quite possible that the hyperactivity associated with residual effects of cannabis treatment (Stiglick and Kalant 1982b) might impair rather than improve performance of a passive avoidance task.

The daily dose of cannabis used in the present study was not extremely high. Rosenkrantz (1983) reported that oral doses of 6–30 mg/kg/day THC in the rat are directly relevant to human consumption, and may be considered as 'moderate' when extrapolated to humans. The residual effects of such treatment on learning can be summarized as follows: Learning is impaired on the Hebb-Williams maze (Fehr et al. 1976), the radial-arm maze (Stiglick and Kalant 1982a), and the DRL-20 task (Stiglick and Kalant 1982b), whereas learning is facilitated on two-way active avoidance (the present experiments). In addition, cannabis-treated rats show residual hyperactivity in an open-field test (Stiglick and Kalant 1982b).

There is a remarkable similarity between these residual effects of cannabis and those of hippocampal lesions. Rats with hippocampal damage are also impaired on the Hebb-Williams maze, the radial-arm maze, and the DRL-20 task (Clark and Isaacson 1965; Rickert et al. 1973; Johnson et al. 1977; Kimble 1978; Olton et al. 1978). Furthermore, such animals show facilitated learning of two-way shuttle box avoidance and hyperactivity in the open field test (Isaacson et al. 1961; Olton and Isaacson 1968; Kimble 1975; Myhrer 1975).

These data lead to the speculation that chronic cannabis treatment might result in hippocampal dysfunction. Future studies should address this possibility directly, by histological, electrophysiological or neurochemical examination of the brains of long-term cannabis-treated animals.

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