Psychopharmacologia (Berl.) 46, 249–254 (1976)

The Effect of Δ^9-Tetrahydrocannabinol and LSD on the Acquisition of an Active Avoidance Response in the Rat

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Received December 10, 1974; Final Version June 16, 1975

Abstract. The course of active avoidance learning of rats in a symmetrical Y-maze under the influence of 1, 3, and 9 mg/kg of Δ^9-THC i.p., and 5, 20, and 100 µg/kg of LSD was investigated.

Δ^9-THC in a dosage of 1 mg/kg had no effect on avoidance learning. Three and to a lesser extent 9 mg/kg produced more rapid learning with a significantly better performance. Learning under Δ^9-THC proved to be state-dependent. The withdrawal of Δ^9-THC caused a decrease in the avoidance rate, which was dependent on the dosage. Upon renewal of the THC doses, the animals reattained their earlier performance. In the course of the experiment there was rapid tolerance development, especially of the sedative properties of THC.

LSD retarded the rate of acquisition of the active avoidance response. Whereas the control animals displayed over 80% successful active avoidance from the 14th session onwards, this was achieved by the LSD groups only after the 20th session. However, in contrast to the control group the LSD animals were able to increase their avoidance rate to over 90%, and this was maintained to the end of the experiment (a total of 24 sessions with LSD). The sudden withdrawal of LSD produced a fall in avoidance rate, which was dependent on the previous training dosage; as with Δ^9-THC state-dependent learning can also be assumed for LSD.

Key words: Δ^9-Tetrahydrocannabinol – LSD – Avoidance response – Rats – State dependant learning – Tolerance – Withdrawal.

Introduction

Information found in the literature concerning the influence of psychotropic substances on conditioned behaviour is often contradictory. Both Δ^9-THC and hashish have been reported to impair performance of previously learned behaviour, and can, depending on the dosage, suppress it completely (Boyd *et al.*, 1963; Grunfeld and Edery, 1969; Carlini *et al.*, 1970; McMillan *et al.*, 1970; Bueno and Carlini, 1972). Grisham and Ferraro (1972) demonstrated that Δ^9-THC has a biphasic effect, *i.e.* in low doses it improves performance, but in high doses impairs it.

Concerning the acquisition of conditioned behaviour under Δ^9-THC, there are reports showing a facilitation of learning ability (Carlini and Kramer, 1965; Barry and Kubena, 1971; Herring, 1972; Pirch *et al.*, 1972), and others in which a negative influence is described (Orsingher and Fulginiti, 1970; Henriksson and Järbe, 1971).

Under the influence of LSD the performance of previously conditioned behaviour is, in general, improved (Täschler *et al.*, 1960; Bignami *et al.*, 1965). Acquisition according to Banerjee (1971) and Domino *et al.* (1965) is impaired under the influence of LSD; however, according to Bignami (1972) it is improved.

Obviously results concerning drug influence on the acquisition of conditioned behaviour depend to a great extent on the experimental method. In order to obtain comparable data, it is advisable to test the different drugs using the same method and the same species of animal. This report describes the behaviour of rats during the acquisition of an active avoidance response under the influence of Δ^9-THC and LSD. As these drugs are at the moment the psychedelics most widely used by young people, the result might be of interest in understanding their problems of drug influence on learning and intellectual development.

Methods

A. Conditioning Apparatus

The experiments were conducted using a symmetrical Y-maze (Fig. 1). The apparatus, constructed of transparent Plexiglas, consisted of three compartments of equal size (for dimensions of the chambers, see Fig. 1), arranged in a symmetrical shape. Each chamber had a separate chargeable grid floor and a lid on the roof to allow the animals to be moved in and out of the apparatus with ease. Each compartment contained a small light (1.4 W) fixed to the back partition. A buzzer was situated at the center of the maze. The current was scrambled and administered across a resistance of 200 kΩ to the

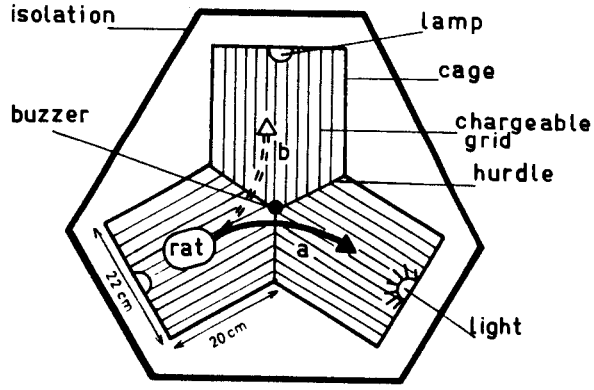

Fig. 1. Top view of symmetrical Y-maze for conditioning of rats. Dimensions in cm, diameter of grid bars 3 mm, interbar distance 13 mm. Rat moving to lighted compartment (*a*) or to dark compartment (*b*)

grating, and adjustable by a Variac (0–350 V) transformer. The animals could be observed by means of a mirror situated above the apparatus.

B. Animals

For the THC experiment 44 albino rats (strain CFN COBS), and for the LSD experiment 34 Wistar rats from an inbred strain were used. The rats were all males and were about 6 weeks old with a weight of 140–160 g when the experiment was started. They were kept either in twos or threes in macrolon cages in an air-conditioned room which was kept at a temperature of 22° C. Food and water were available *ad lib.* The reversed day-night rhythm consisted of light from 7 p.m. – 7 a.m. and in darkness from 7 a.m. – 7 p.m.

C. Drugs

THC Experiment. (–)-trans-Δ^9-THC (Public Health Service, U.S.A.) was dissolved in polyethylene glycol 300 (PAEG 300) so that the injection volume was 0.5 ml/kg rat.

LSD Experiment. LSD-tartrate (Sandoz AG) was dissolved in saline solution. The injection volume was 1 ml/kg rat. Both substances were administered i.p., Δ^9-THC 1 h and LSD 30 min before each conditioning experiment.

D. Conditioning

THC Experiment. During the first 14 days the young rats were familiarized with their new surroundings, and handled (held and stroked) for several minutes daily. Then on 3 successive days the frequency with which an animal cleaned itself, reared, and moved from one compartment of the Y-maze to another within 5 min was counted. On the basis of these measurements 4 groups of 11 rats per group were formed, matched on the basis of spontaneous activity level.

Groups II, III, and IV were the THC groups and group I served as the control group. The task to be learned by the rats was conditioned avoidance reaction (CAR). At the beginning of the experiment one of the two empty compartments was lit, and a buzzing noise began. After 2.5 s (from the 10th session on 5 s), during which time only the light and sound conditioned stimuli (CS) were given, the grating in the two unlit compartments was electrified for 3 min. The rats learned at first to go to the lit compartment be escaping the unconditioned shock stimulus (UCS), and later to avoid the current by moving to the correct compartment upon onset of the conditioned stimuli. The buzzer was switched off as soon as the animal made the correct response, whereas the light was left on until the end of the trial. Up to the 10th session there was a pause of 10 s between the trials, later the trials were carried out immediately one after another. The strength of the current was individually adjusted so that the animal concerned clearly reacted to the shock, but did not cry out with pain. The target compartment was chosen by use of a random numbers table.

The number of trials per session was increased from 15 in the first session, through 20 and 25, to 30 in the 4th and following sessions. The number of times an animal actively avoided the current per session was established. The percentage number of correct responses per session provided the so-called avoidance rate. Whether the animal went directly to the correct compartment or made a detour *via* the second unlit compartment, was not taken into consideration.

From the first session onward, group I (controls) received an i.p. injection of 0.5 ml/kg of PAEG 300 1 h before the session and groups II, III, and IV received Δ^9-THC in doses of 1, 3, and 9 mg/kg respectively. The sessions took place twice a week, on Tuesday and Friday, always at the same time of day.

After the 4th session the animals which did not avoid the current were discarded so that 9 animals remained in groups I, II, III, and 10 in group IV. Altogether 26 sessions were carried out with Δ^9-THC. On the 26th and 27th session days all groups were injected with the solution only. Prior to the 28th session the THC groups were injected once more with Δ^9-THC.

LSD Experiment. Conditioning was achieved using the method described for Δ^9-THC, but the conditioned stimulus consisted only of the light. The division into groups and the elimination of the animals not capable of learning the avoidance task were decided in the same way as in the THC-experiment.

From the first session onward, group I (control $n = 7$) was given 1 ml/kg of NaCl-solution, and groups II, III, and IV ($n = 8$ per group) received LSD in doses of 5, 20, and 100 µg/kg (i.p. 30 min before the trial). The animals were trained twice a week. On the 24th day of the experiment the LSD groups were given the solution only, instead of LSD. The 25th session was carried out after renewed administration of LSD. There were then 4 more sessions before which all groups were injected with the solution only.

E. Statistics

Each THC group was compared with the control group using a two-way analysis of variance, whereby the learning curve from the 1st to the 20th day (in group IV from the 5th to the 20th day, in addition) was analyzed. The original percentages (x) were transformed to $y = \text{arc sin } \sqrt{\frac{x}{100}}$ to stabilize the variation. In addition, for every experimental day the mean avoidance rates of the THC groups were compared with those of the control group by t-tests.

The performance of the LSD groups was compared with the control group using the Mann-Whitney-test. For the withdrawal experiment (23rd, 24th, and 25th days of experiment)

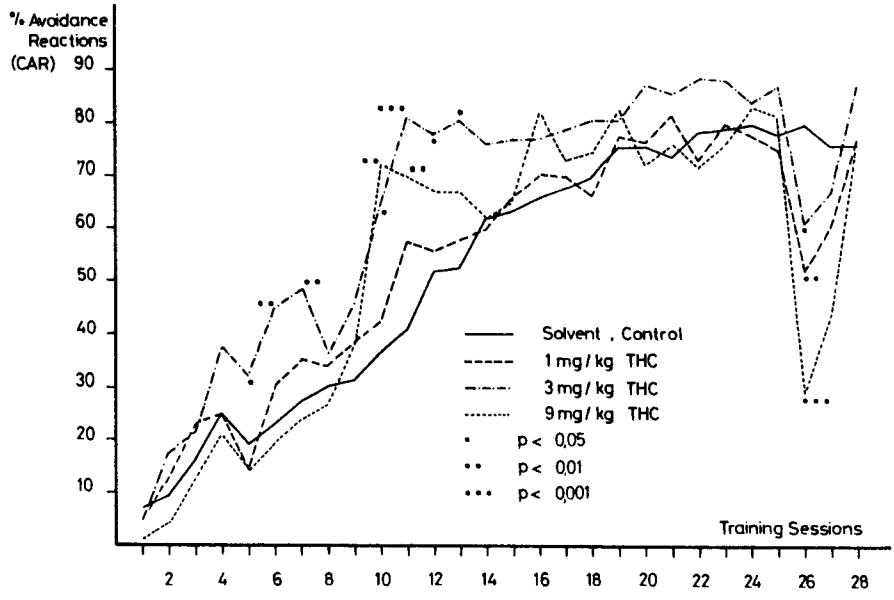

Fig. 2. Acquisition of a conditioned avoidance reaction (CAR, ordinate) during consecutive training sessions (abscissa, see text). The regular i.p. injections of THC 1 h before the sessions were omitted before sessions 26 and 27, but resumed in session 28

the 3 LSD groups were pooled together and the overall mean avoidance rates, with and without LSD, were compared (Wilcoxon-test).

Results

Δ^9-THC (see Fig. 2). The 1 mg group did not differ from the control group as far as the overall avoidance rate was concerned.

In contrast the 3 mg group showed, even in the 5th session, a significantly higher rate of active avoidance which rose suddenly to over 80% on the 10th and 11th days of the experiment. The variance analysis showed that, for the period from the 1st to 20th experimental days, avoidance learning of the 3 mg/kg THC group was significantly better than that of the control group ($P < 0.001$).

The 9 mg/kg group also showed a significantly better avoidance rate on the 9th, 10th, and 11th days. The variance analysis for the period from the 1st to the 20th day, however, did not show an overall significant difference from the control group. For the period from the 5th to the 20th day the performance of the 9 mg/kg THC-animals was significantly better by $P < 0.01$ to 0.05. The avoidance rate of the 9 mg group fluctuated considerably from session to session, only on certain days was it higher than 80%.

The withdrawal of Δ^9-THC on the 26th day of the experiment produced in all THC groups a marked drop in the avoidance rate which was most pronounced in the 9 mg group. In the second session without THC the number of active avoidance attempts increased again noticeably. With Δ^9-THC (28th day of the experiment) the THC animals reattained the avoidance rate they had before the withdrawal.

In comparison with the control group the animals in the 1 mg group seemed more excited and made more errors by frequently running from the correct compartment again. In contrast the animals from the 3 mg group were sedated and appeared sleepy at the beginning of the experiment (*i.e.*, 1 h after injection), but responded quickly and often irritatedly to the electric current. The animals were however never impeded enough to prevent them from escaping the current. This group efficiently learned the appropriate flight and avoidance reactions. They did not display the same irritation which led to mistakes in the 1 mg group.

In the 9 mg group irritation was visible shortly after injection. Two animals reacted aggressively and assumed a fighting posture against one another. After 1 h the animals were heavily sedated and suffering from ataxia, but nevertheless displayed a high sensitivity to stimulation, *e.g.*, they squealed when picked up. Often they were not capable of avoiding the electric shock and reacted by jumping in an uncoordinated manner.

In all groups the gross behavioral effects of THC gradually became attenuated with repeated injections, even after the 5th THC administration. After approximately 20 injections the animals appeared to be completely tolerant to THC.

During the course of the experiment 6 animals died: 3 from the 9 mg group, 2 from the 3 mg group, and 1 from the 1 mg group. On examination atrophy of the spleen and liver, symptoms of peritonitis, as well as concretions of organs were found. At the end of the experiment the weight of the THC animals was significantly lower than that of the control group (particularly the 9 mg animals).

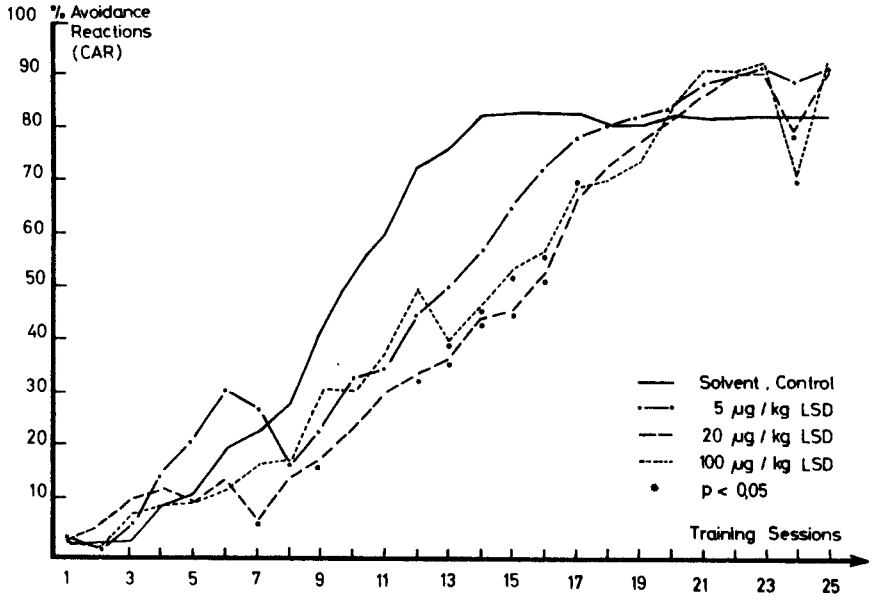

Fig. 3. Acquisition of avoidance reaction (CAR) under different doses of LSD given i.p. 30 min before sessions. Withdrawal before session 24

LSD (see Fig. 3). The 3 LSD groups, and in particular the 5 µg/kg group, surpassed the control group for a short time in avoidance learning, but not significantly. From the 6th to the 8th session onward the LSD animals fell back noticeably, and the avoidance rates of the 20 µg and 100 µg groups were significantly lower than that of the control group. The control group reached a constant level of 82–84% correct responses after the 13th day of the experiment. The LSD groups only attained this percentage 5–7 sessions later; however, this then increased to 93%. The better performance displayed by the LSD groups in this phase was not significant.

The withdrawal of LSD on the 24th day of the experiment produced in all LSD groups a decrease in the avoidance rate, which was dependent on the previous training dosage (not significant in the 5 µg/kg group). The collective decrease in avoidance rates of the 3 LSD groups upon LSD withdrawal with and without LSD, was significant at $P < 0.05$. On receiving LSD again the animals attained their previous performance level once more. After the final withdrawal of LSD the collective avoidance rate of the LSD groups did not reach the level (over 90%) attained prior to withdrawal, even after several sessions without LSD *i.e.* the LSD groups taken together attained only 84% correct responses, which is exactly the value for the control group.

Discussion

THC-Experiment. Our experiment showed that the acquisition of an active avoidance response in a Y-maze was not influenced by Δ^9-THC in doses of 1 mg/kg, but was facilitated with doses of 3 and 9 mg/kg. Whereas Pirch *et al.* (1972) also observed facilitation in a similar experiment, the result is not consistent with that of Henriksson and Järbe (1971), although the experimental methods used by these authors were very similar to ours. Over 6 days Henriksson and Järbe gave rats 7.5 mg/kg of Δ^9-THC i.p., always 30 min before the sessions. On the 6th day the THC animals displayed a significantly lower avoidance rate in the shuttle box than the control animals.

These diverging results can above all be attributed to the tolerance development which is not greatly pronounced even after the 6th THC injection (Henriksson and Järbe, 1971). As can be seen from Fig. 2, the avoidance rate in our experiment rose rapidly from the 9th to the 11th session days onward, at a time when clear signs of a development of tolerance to the suppressing THC properties became visible. This confirms the evidence of Goldberg *et al.* (1973) and Potvin *et al.* (1972). According to them the stimulating effect of Δ^9-THC, which has a positive influence on performance, becomes increasingly more evident in the course of the tolerance development.

In the 9 mg group the suppressive effects of THC were pronounced; during the sessions the arousal normally induced by the electric shock was attenuated. Barry and Kubena (1971) speak of "an arousal reaction" which is brought about by the shock and works against the sedative state.

The more rapid acquisition of the avoidance response by the animals of the 3 mg group could be attributed to the fact that the animals, as a result of increased sensitivity to stimulation, reacted much

more strongly to light, noise, and current and because of this discovered the safe compartment more quickly.

The experiment confirmed that learning under THC is "state-dependent" or dissociated (Henriksson and Järbe, 1971; Järbe and Henriksson, 1973). In all groups after sudden withdrawal of Δ^9-THC, *i.e.* the substance under whose influence the animals had acquired the avoidance response, there was a drop in performance which was most pronounced with the highest dosage.

Dissociated learning was seen in animals which were apparently tolerant ("behaviorally" tolerant) to depressant effects of Δ^9-THC, *i.e.* Δ^9-THC continued to function as a discriminative stimulus (see Bueno and Carlini, 1972; Carder and Olsen, 1973). The chronic toxicity of Δ^9-THC which became more distinct during the course of the experiment and which was accompanied by anorexia and organ atrophy, was also observed by other authors (Phillips *et al.*, 1972). According to Thompson and Braude (1972) and Manning (1971) this also occurred with peroral administration of Δ^9-THC. The peritonitis could be attributed to the i.p. injection (see Manning *et al.*, 1971). The animals in the control group displayed a normal gain in weight and none died during the experiment; any toxic effects of the solution (PAEG 300) can therefore be ruled out (see Smyth *et al.*, 1950, 1955).

LSD Experiment. The experiment showed that 5 µg, 20 µg, and 100 µg/kg of LSD slowed the acquisition of an active avoidance response, but that the LSD animals gave a better end result than the control animals, which was not significant. The slower increase in avoidance learning by the LSD animals could be explained by the discovery of Key (1960, 1961, 1964), Key and Bradley (1960), and Bradley and Elkes (1959), that LSD lowers the threshold for response to stimuli, resulting in an inability to distinguish between important and unimportant stimuli.

LSD apparently interferes with the mechanism for the selection and integration of afferent information. In other words, LSD facilitates in small doses the processing of sensory information, but leads to "stimulus generalization" if two similar stimuli are present at the same time, *i.e.* an incapacity to distinguish between stimuli.

The fall in performance between the 6th and 8th sessions could possibly be connected with the development of tolerance. According to Appel (1967) tolerance occurred only after 7–8 days in those animals given 130 µg/kg of LSD. That an LSD effect was still present even after 23 sessions is shown by the fall in performance when LSD was suddenly withdrawn on the 24th day of the experiment, and by the restoration of performance with renewed LSD administration. The acquisition of the CAR task under LSD was shown, as under Δ^9-THC, to be "state-dependent".

The fact, that even after several sessions without LSD some animals from the LSD groups (1 or 2 animals per group) did not achieve the high level of performance they did under LSD could be an indication that more "stupid" animals reach a possibly better level of performance with LSD. The LSD groups thus reached a mean active avoidance rate of 94%, above the control group. In the control group also the average result was depressed by 2 animals which constantly achieved an avoidance rate of only 50–70%. It may be assumed that LSD does not have the same effect on "stupid" and "intelligent" animals, a phenomenon which is also known of amphetamine. Slow animals react better with amphetamine, whereas lively animals are aroused more and make errors. Inversely, a compound with sedative properties can increase the performance of aroused animals and impair that of slow animals.

Acknowledgements. We gratefully acknowledge the financial help of the Swiss National Research Foundation (project No. 3.692.71), the valuable advice of Dr. J. Huston, and the skillful help of Miss E. Pichler and Mrs. L. Körber.

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