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Original Investigations

Comparison of Δ^9-THC, LSD-25 and Scopolamine on Non-Spatial Single Alternation Performance in the Runway

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Abstract. Rats were trained in a straight runway on a non-spatial single alternation (NSSA) which involved presentation of reward (R) and nonreward (N) in a fixed repeating sequence (*i.e.* R—N—R—N . . .). Patterned running results since rats learned to run fast on R trials and slow on N trials. This task can be regarded as an animal analog of the goal directed serial alternation task employed with humans. After patterned running was well established the effects of graded doses of Δ^9-THC, LSD and scopolamine were delineated. Although all drugs modified alternation performance, each agent produced distinctly specific effects on the different components of NSSA. Δ^9-THC disrupted alternation by decreasing running speed on R trials and increasing running speed on N trials. Lower doses of LSD increased running speed on N trials while leaving R trial speeds unchanged. At the highest dose, LSD decreased running speed on R trials while leaving N trial speeds only slightly elevated from baseline. Scopolamine disrupted alternation solely by decreasing speed on R trials. The results were discussed with reference to the effects of these drugs on internal inhibition, registration and recall of internal cues and timing behavior.

Key words: Δ^9-THC — LSD — Scopolamine — Non-Spatial Single Alternation — Patterned Running — Inhibition — Internal Cue — Timing Behavior.

Although a number of studies have shown that marihuana and tetrahydrocannabinols (THC) markedly impair certain aspects of memory functioning in man (Abel, 1971; Miller *et al.*, 1972; Weil and Zinberg, 1969), only a few animal studies have been directed toward delineating the effects of cannabinoids on memory in animals. In monkeys, Scheckel *et al.* (1968) found that both Δ^8 and Δ^9-THC disrupted performance on a delayed matching task, and Zimmerberg *et al.* (1971) have shown that marihuana smoking impairs matching performance, while Δ^9-THC (orally) impairs performance on an oddity problem. Even fewer data are available concerning the mechanism through which cannabinoids exert their action on memory. However, one interesting hypothesis has been offered by Melges *et al.* (1970, 1971), based

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on studies with a "goal directed serial alternation" arithmetic task (GDSA) in man. After administration of a marihuana extract (calibrated for Δ^9-THC content), these investigators found that the impaired performance on the GDSA observed could be interpreted as a consequence of "temporal disintegration"—*i.e.*, disruption of the temporal organization of the sequential mental operations required for reaching a goal.

Development of an animal model of the GDSA would be of great value for further investigations on brain mechanisms through which cannabinoids impair memory functioning. As a first step in that direction, we have explored the possibility of employing a non-spatial single alternation (NSSA) paradigm in a straight runway for use with rats. NSSA involves the presentation of reward and non-reward in a fixed repeating sequence (e.g., R—N—R—N, etc.). After an extended period of training, an animal such as the rat learns to run fast on R trials and slowly on N trial (fast after N, and slowly after R). No exteroceptive cues are available to distinguish R from N trials. According to Capaldi (1967), on an alternating schedule of reinforcement, reward and non-reward occasion distinctive internal stimuli, S^R and S^N. Following non-reward, S^N comes to be linked, through conditioning, to the instrumental response on the next trial, because that trial is always reinforced. However, because S^R is always followed by a response that is never rewarded, inhibition accrues to S^R, resulting in a response decrement. Thus, in the presence of S^R an animal will run slowly because this internal cue predicts that non-reward is due. In the presence of S^N, an animal will run fast because S^N signals that reward will follow. Thus, the animal learns to "remember" the sequential temporal organization of R and N trials, in relation to a "goal" (food).

However, use of the NSSA requires consideration of the possibility that, like anticholinergic drugs (Carlton, 1963, 1968) Δ^9-THC might produce changes in behavior that could be ascribed not to impairment of memory, but to "disinhibition" of behavior controlled by non-reinforcement. Jaffe and Baum (1971) reported increased resistance to extinction of a conditioned avoidance response in rats treated with hashish resin, and Brown (1971) found that in mice Δ^8-THC inhibited the "latency attenuating (habituating) effect of previous exposure" to an experimental chamber. More specifically, it is conceivable that Δ^9-THC, like anticholinergic drugs, may selectively disrupt "internal inhibition" since in the case of anticholinergic drugs, the studies of Heise and Lillie (1970) and Heise *et al.* (1971) indicate that such agents have a distinct effect on behaviors that are under internal inhibitory control (*i.e.*, behaviors that had come to be inhibited in the absence of any external stimulus signaling that a period of non-reinforcement was due). Anticholinergic drugs were found to be more consistently disruptive of inter-

nally, rather than of externally controlled inhibiton. Apparently, LSD-25 also disrupts internal inhibition, since Key and Bradley (1960) and Key (1964) reported that in cats, this psychotomimetic agent delayed extinction of "arousal" responses to non-reinforced stimuli when the drug was given prior to commencement of the extinction procedure, and restored "arousal" responses when the drug was given after extinction had been accomplished.

Therefore, to control for possible disruptive effects of Δ^9-THC on "internal inhibition" (the consequences of which might appear on the N trials in the NSSA), comparisons were made of Δ^9-THC dose-response curves (for both the R and the N trials) with those of scopolamine (an anticholinergic drug) and LSD-25.

Method

The *Ss* were 15 male hooded rats (Purina Laboratories) 90—100 days of age at the beginning of the investigation. All *Ss* were individually housed with free access to food and water for one month prior to the initiation of the study.

Apparatus

The apparatus was a straight, grey plywood alley 200 cm long $\times$13 cm wide $\times$30 cm deep. A manually operated plexiglass guillotine door was located approximately 32 cm from the goal end of the alley. This door demarcated a goal section and was used to prevent retracing. A brass cup attached to the end wall of the goal box served as a receptacle for food reinforcement. Running time was recorded with 0.01 sec. Lafayette electric timers triggered by photocell relays along the alley. A background white noise of 70 db masked extraneous noise.

Procedure

One week prior to training, *Ss* were put on a 23 h food deprivation schedule and handled each day. All *Ss* then received training on the runway for continuous food reward (R—R—R—R . . .) for a total of 30 trials at 5 trials per day, following which they were switched to an alternating schedule of reinforcement (R—N—R—N) for a total of 180 trials at 9 trials per day. On any trial in which an animal failed to break the final photobeam within 180 sec, the experimenter gently forced it into the goal box and through the last photocell. The intertrial interval was 15 sec in both the training and experimental phases of this study. Reinforcement consisted of two 250 mg. Noyes pellets which were present on all R trials. When patterned running was well established, graded doses of LSD, scopolamine and Δ^9-THC were administered to test for their effect on alternation. Following each dose level of any agent responding was allowed to return to baseline before the next dose was given. A 7 to 10 day interdose interval proved adequate for this purpose. Running speeds were also measured during this interval.

Drugs

The following doses of each drug were used: LSD (0.016, 0.048, 0.14 mg/kg), scopolamine (0.0125, 0.05, 0.1 mg/kg), and Δ^9-THC (2, 3, 5 mg/kg in 1% Tween 80-water). All drugs were prepared as active base. Details on the preparation of Δ^9-THC

have been presented elsewhere (Drew *et al.*, 1972). Saline and 1% Tween 80-water were employed as vehicle substances. All doses were given in an increasing order of strength, and all doses of one drug were given before the lowest dose of the next drug was administered. In an effort to control for possible drug interactions, a 14 to 20 day interval separated the administration of different drugs. THC was the first drug administered followed by LSD and scopolamine. All drugs were given intraperitoneally 30 min prior to testing. The duration of running time on any given treatment day for an individual animal was 15—20 min. Tween or saline were administered during a session 1—2 days prior to treatment with the lowest dose of a given drug. Scopolamine doses were chosen on the basis of pilot observations in our laboratory, while the choice of the THC doses and post-injection interval were based on a previously published open field study (Drew *et al.*, 1972). The rather narrow range of Δ^9-THC doses was chosen because doses much higher than 5 mg/kg in naive rats have been found to produce moderate to marked cataleptoid reactions and ataxia (Grunfeld and Edery, 1969). Doses of LSD similar to those used in the present investigation have been employed in the study of behavior maintained by various schedules of reinforcement (Appel, 1968).

Statistical Evaluation

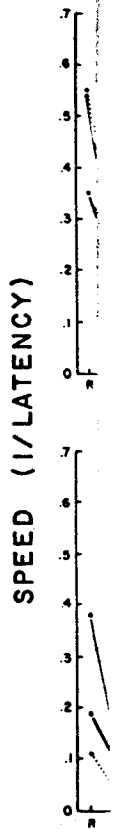
All running times (latencies) were converted to speed scores (1/latency). The effects of each drug on NSSA were evaluated separately by analyses of variance performed on the mean running speeds for each treatment day. An analysis consisted of 3 within subject factors including a dose effect, an effect for reinforced versus non-reinforced trial differences (R—N) and a blocks effect (2 trial blocks including one N and one R trial). Thus, there are 4 trial blocks on any treatment day. The first R trial in each condition was not included in the analysis for computational reasons and because the first R trial is actually a cue-producing trial. Running speeds on this trial were not reflective of speeds on other R trials.

During the course of the study a number of animals were dropped due to sickness or death. Under THC treatment 15 *Ss* were run. Under LSD and scopolamine the number dropped to 11 and 9 respectively. The loss of these *Ss* resulted in a mild improvement in baseline difference scores.

Results

The effects of Δ^9-THC, LSD and scopolamine on NSSA are graphically presented in Figs. 1 and 2. The upper left quadrant of Fig. 1 illustrates the typical "sawtooth" curve characteristic of NSSA under control conditions. In each quadrant, group mean alternation performance and the alternation performance of two individual *Ss* are presented for various treatment conditions. The dose-response effects for each of the drugs on group alternation performance (R—N), R and N trial speeds, and mean running speed are presented in Fig. 2.

Δ^9-THC. Treatment with THC resulted in a lowering of mean running speed and a dose-dependent reduction in alternation (R—N) performance (Fig. 2). This was reflected in the significant dose-effect ($F = 4.63, df = 3/42, p < 0.01$) and dose XR—N interaction ($F = 68.13, df = 3/42, p < 0.001$). Individual comparisons indicated that a signif-



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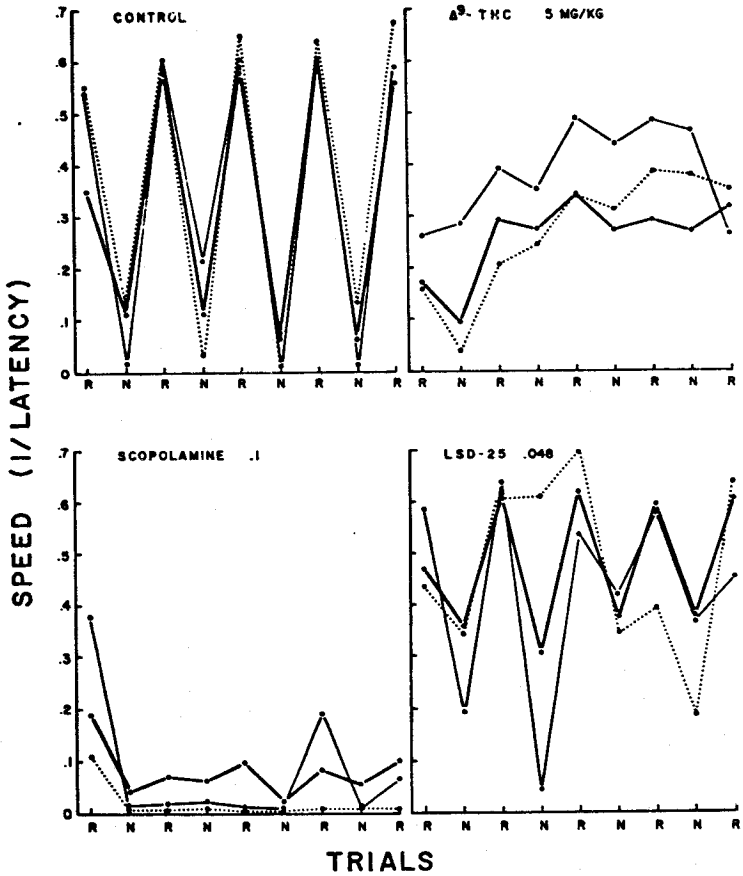

Fig. 1. Comparison of Δ^9-THC, scopolamine and LSD-25 on patterned running in the rat. Upper left quadrant shows typical control "sawtooth" pattern of NSSA obtained under no-treatment, saline or 1% Tween 80-water conditions. Note fast speed on rewarded (R) and slow speed on non-rewarded (N) trials. Remaining quadrants show effects of selected dose levels of Δ^9-THC, scopolamine and LSD on NSSA. All doses are expressed as mg/kg body weight. In all quadrants, *heavy* dark lines represent the group mean pattern, whereas, the *light* dark lines and the dotted lines represent typical responding from two individual rats

icant drop in alternation from control levels occurred with the initial dose of THC ($p < 0.001$). While *Ss* under the 2 mg/kg condition displayed significantly higher alternation scores than under the 5 mg/kg condition, the 2 and 3 and 3 and 5 mg/kg conditions did not differ from each other. The reduction in alternation appeared to be linearly related to dosage.

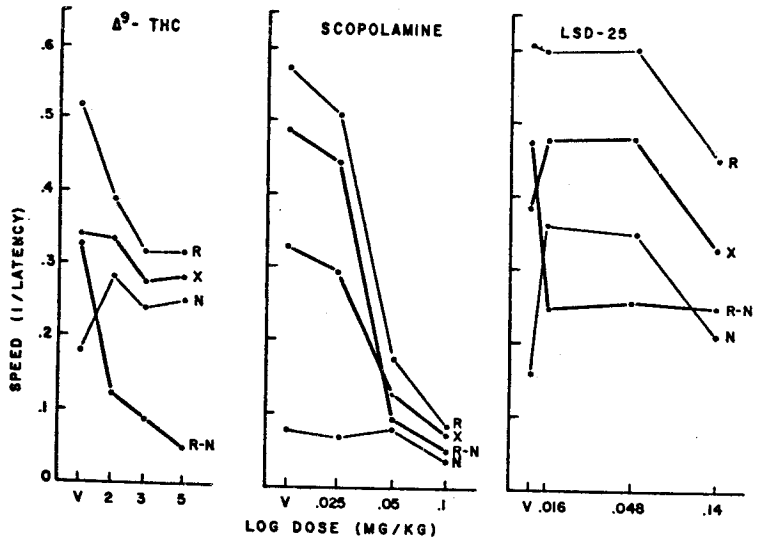

Fig. 2. The effect of graded doses of Δ^9-THC, scopolamine, and LSD-25 on specific components of NSSA responding. Mean running speed on reinforced and non-reinforced trials under each treatment condition are designated by the letters R and N respectively. The difference in running speed between R and N trials is a measure of alternation (R—N). A higher score on the ordinate indicates superior discrimination of R and N trials (i.e. faster running on R trials and slower running on N trials). Mean running speed (X) was calculated by averaging running speed on R and N trials. The letter V refers to vehicle. For Δ^9-THC the vehicle employed was a volume of 1% Tween 80-water which would theoretically deliver 5 mg/kg Δ^9-THC. Saline was employed as vehicle for both LSD-25 and scopolamine, the amount administered being equal in volume to the largest dose of either drug

Generally, the reduced alternation produced by THC appears to be due to two factors, decreased running speed on R trials and increased running speed on N trials. On R trials, there was a significant drop in running speed from baseline with the 2 mg/kg dose. Alternation scores also differed under the 2 and 3 and 2 and 5 mg/kg doses ($p < 0.01$). The 3 and 5 mg/kg conditions did not differ. On N trials, a significant increase in running speed occurred from baseline ($p < 0.001$), with no differences existing between individual doses.

LSD. The dose-response curve for LSD in Fig. 2 indicates that a decrease in alternation occurred with the initial dose of LSD. Comparisons of the individual alternation means indicated that while difference scores (R—N) were decreased significantly from control levels at each of the three LSD doses ($p < 0.001$) this decline remained constant across doses. Mean running speeds were faster than those

observed on baseline days at the two lower doses, but speeds decreased below baseline at the higher dose. This accounts for the significant dose effect ($F = 12.15$, $df = 3/30$, $p < 0.001$). Of particular importance was the significant dose X R—N interaction ($F = 5.91$, $df = 3/30$, $p < 0.005$). Although alternation (R—N) was invariant under the different doses of LSD, running speeds on R and N trials were differentially affected at each dose. Under the two lower doses, R speeds were unchanged, while a significant decrement from baseline occurred with the highest dose ($p < 0.001$). Speeds on N trials were significantly increased over baseline under the first two doses and were decreased from this plateau at the highest dose level to near control levels. Thus, decreased alternation at the two lower doses appears to be due to accelerated running on N trials, while at the highest dose decreased alternation was due mainly to altered running speed on R trials.

Scopolamine. Fig. 2 shows that scopolamine produced effects on alternation which are distinctly different from those observed under LSD or THC. A steep reduction in mean running speed and alternation occurred as the dosage of scopolamine increased. The reduction in mean running speed was reflected in a significant dose effect ($F = 27.54$, $df = 3/24$, $p < 0.001$). The figure clearly indicates that the decline in alternation as well as mean running speed is due to depressed R trial speeds. This was reflected in the significant dose X R—N interaction ($F = 48.98$, $df = 3/24$, $p < 0.001$). Speed on N trials remained unchanged from baseline.

The decreased running speed on R trials appears to be largely indicative of a decrease in appetitive motivation. Most *Ss* failed to complete the consummatory response on R trials at the two higher dose levels of scopolamine. It was also noted that following scopolamine injections, *Ss* took 1—2 days longer to return to control levels of responding than under LSD or THC.

Discussion

All three drugs disrupted or at least modified "sequential alternation", but in markedly different ways. In the case of Δ^9-THC, the linear dose-response reduction in alternation was attributable to an increase in running speed on N trials, and a decrease in running speed on R trials. LSD-25 appeared to increase running speed on the N trials at the two lower doses without affecting running speeds on the R trials, but at the highest dose used, LSD-25 increased running speeds only slightly on the N trials, while depressing running speeds on the R trials below baseline values; across dose, the consequent decrease in alternation remained invariant. Scopolamine, on the other hand, disrupted alter-

nation solely by decreasing running speeds on the R trials; running speeds on the N trials were not altered.

If only changes in running speeds are considered, then the pattern of effects of Δ^9-THC are reminiscent of the "paradoxical" effects of amphetamine upon high and low rates of operant responding (decrease in high-rate, and increase in low-rate responding, Dews, 1958). At best, however, such a "law of initial values" is only a description, not an explanation of the phenomenon, and consideration of other changes in the behavior of rats treated with Δ^9-THC suggests that the mechanisms involved in the generation of such apparently similar response-patterns are not the same for Δ^9-THC and amphetamine. Thus, the running behavior of rats on N trials in the absence of drugs is normally characterized by what may be termed, "bursting" and "pausing"; *S* runs and then lingers, alternately. This behavior is not seen on R trials. However, after administration of Δ^9-THC, "pausing" behavior generalized to R trials, and "bursting" became more prominent on N trials. Furthermore, the analogy between running speed in the NSSA situation and rates of responding in an operant situation may be misleading, inasmuch as Frankenheim *et al.* (1971) reported that while Δ^9-THC, in doses similar to those employed in the present study, decreased bar pressing rates in an FR component of a multiple schedule of reinforcement, the drug had little effect on an FI component. Barry and Kubena (1970) found that Δ^9-THC, in doses up to 8 mg/kg, failed to decrease bar pressing rates in rats on a continuous avoidance task.

With regard to the possible mechanisms of action of THC, it appears that selective "disinhibition" of inhibitory responses (to the postulated inhibitory internal S^R cue) cannot explain all the data observed in the present studies, since Δ^9-THC, while indeed increasing running speeds on the N trials, decreased running speeds on the R trials (presumably controlled by excitatory internal S^N cues). Incidentally, it should be noted that while running speeds on the R trials were decreased as a function of dose of Δ^9-THC, the consummatory response was not impaired at any dose. This observation is at variance with studies reporting that THC interferes with food intake, resulting in deficits on appetitively motivated tasks (Manning *et al.*, 1971). Unlike Δ^9-THC, LSD-25, at least at the two lower dose levels, did appear to selectively disrupt inhibitory responses to the postulated internal S^R cues, but surprisingly, scopolamine did not: running speeds on N trials after scopolamine remained unchanged from baseline at all dose levels studied, while running speeds on R trials decreased as a function of dose. However, the failure of scopolamine to exert its expected effects may have been due to the use of a solid food reinforcer; in consonance with

the findings of Olds (1970), we observed that with the highest dose of scopolamine used, no animal finished eating in the goal box (on R trials), and in some cases, *Ss* had to be forced into the goal box.

More compatible with all the data, are hypotheses concerning the actions of Δ^9-THC that are related to disruption of the "temporal organization" required for memory. In this connection, two hypotheses could account for the data: 1. a Δ^9-THC induced deficit in the ability to register or recall the postulated S^N and S^R traces: or 2., Δ^9-THC induced time sense disturbance.

In terms of the first hypothesis, the emergence of behavior which is incompatible with that normally seen on given R or N trials may reflect an indecision with regard to reward expectancy. It is conceivable that the emission of "pausing" and "bursting" behavior on both R and N trials after Δ^9-THC may represent a failure of drug treated *Ss* to register an internal cue *following* a goal box event or a failure to recall the relevant internal stimulus which should be operative *prior* to a goal box event. This explanation would be consistent with the reported deficits in short term memory seen in human subjects treated with marihuana (Tinklenberg *et al.*, 1970; Dornbush *et al.*, 1971). Such does not appear to be the case for LSD-25 or scopolamine, since generalization of "bursting" and "pausing" did not occur after administration of these drugs, and the changes in running speed patterns they produced can be explained on other grounds (see above).

In terms of the second hypothesis, adequate performance on the NSSA task can be viewed as being dependent on the evocation of two incompatible responses which follow each other successively and are therefore appropriate at different points in time. It Δ^9-THC speeds up an internal biological clock resulting in distortion of true time estimation as Melges *et al.* (1970) and Drew *et al.* (1972) have suggested, the sequentially alternating interoceptive cues that had been acquired by conditioning during training, could evoke responses that are appropriate to the cues, but inappropriate for a given point in true time. Resolution of these two alternative hypotheses must await the outcome of further research.

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