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EFFECT OF DELTA-9-TETRAHYDROCANNABINOL ON SPATIAL MEMORY OF THE RAT

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ABSTRACT--Many of the behavioral and physiological effects of marijuana have been documented in several animal species, including humans. However, very little is understood about the mechanism of action of marijuana's chemical components in the brain. Several years ago it was noted that Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive constituent of marijuana, concentrates heavily in the hippocampus. Recently, cannabinoid receptors were discovered in large numbers both there and in the cerebral cortex. Experiments using lesioning techniques and a novel procedure requiring rats to find a hidden goal in a tank of opaque water indicated that the hippocampus may be the seat of spatial memory in the brain. In order to elucidate a basis for a link between Δ^9-THC receptors in the hippocampus and observable cognitive effects, the water-tank procedure was used to test the effect of Δ^9-THC on spatial memory. The results indicate that, at a low dose (2 mg/kg), Δ^9-THC does not hamper spatial memory, but further studies are required to determine whether or not higher doses may.

There is little doubt that the hippocampus is involved in general memory and learning. Douglas (1967), Drew and Miller (1974), Drew et al. (1980), and Zola-Morgan and Squire (1990) have been endeavouring to separate some learning processes into specific categories. Research with single units in the rat dorsal hippocampus (fields CA1 and CA4) and in the dentate gyrus suggests that specific parts of the hippocampus are vitally important to spatial memory and cognition in particular (O'Keefe and Dostrovsky, 1971). It is reported that these units fire in response to a precise spatial orientation, usually in combination with an appropriate sensory stimulus. Some units seem clearly to be involved in the association of location with the occurrence of sensory stimuli, while others signal that the rat is in a specific place in the environment, regardless of its reasons for going there (O'Keefe and Nadel, 1974). Rats with hippocampal lesions exhibit a significant deficit in spatial memory when compared to rats with superficial neocortical lesions, sham-operated rats, and untreated rats (Morris et al., 1982).

The hippocampus also seems to be a major site of marijuana activity, exhibiting high binding and retention levels of Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive ingredient of marijuana (Essman, 1984; Hampson et al., 1989). Specific cannabinoid receptors were identified in 1990 and were found to be densely concentrated in the hippocampus (Bidaut-Russell et al., 1990; Matsuda et al., 1990) and the dentate gyrus (Howlett et al., 1990). There is strong evidence that Δ^9-THC affects behavior patterns associated with spatial memory and that it alters electrical activity of cells in the CA1 dorsal hippocampus and dentate gyrus (O'Keefe and Dostrovsky, 1971; Drew et al., 1980; Weisz et al., 1982; Campbell et al., 1986*a*).

The effects of Δ^9-THC on spatial memory have been investigated only partially. Essman (1984) reported that rats exposed to marijuana smoke showed a deficit in the retention of a conditioned passive avoidance response, which could also be considered a spatial memory task, since it requires the association of an electrical shock with a location. Aircraft pilots performing in a flight simulator were shown to have impairments on a simple landing procedure after being exposed to Δ^9-THC. The deficits were significant in "number and size of aileron changes, size of elevator changes, distance off-center on landing, and vertical and lateral deviation on approach to landing" (Yesavage et al., 1985). The deficits persisted 24 h after exposure to marijuana, which correlates with Essman's (1984) discovery of relatively large concentrations of Δ^9-THC in the rat hippocampus 24 h after cannabinoid administration.

The tasks performed by the intoxicated pilots could require the use of spatial cognition to visualize the position of the craft in space. If Δ^9-THC hampers spatial cognition, then it is likely to cause a deficit in place-navigation similar to the deficit exhibited in hippocampectomized rats. The purpose of our study was to test the possibility that Δ^9-THC affects the hippocampus by altering spatial cognition mechanisms.

MATERIALS AND METHODS

Apparatus and Subjects--The water-tank procedure that was developed by Morris (1981) to perform spatial-memory experiments was deemed appropriate for our experiments. This technique requires rats to remember the fixed location of an escape platform hidden just below the surface in a tank of opaque water. It is ideal for testing the effects of Δ^9-THC on spatial information processes because it separates spatial learning from other types of learning. Mazes, for instance, can complicate the issue of spatial learning because they offer a series of choicepoint decisions as the rat weaves its way through. Furthermore, the water-tank task eliminates the necessity of a food reward, a particularly important feature for our study since cannabinoids have been reported to increase appetite and make food more appealing (Aulakh et al., 1980).

Forty-three male Sprague-Dawley rats weighing from 200 to 220 g were used. Each was randomly assigned to one of four groups: 1) received Δ^9-THC and required to locate invisible platform; 2) received placebo and required to locate invisible platform; 3) received Δ^9-THC and required to navigate to visible platform; 4) received placebo and required to navigate to visible platform. Each animal was allowed food ad lib throughout, except during the testing periods.

The tank used for the experiment was a rough, plastic wading pool. Its diameter was 1.55 m, and it was filled with water to a depth of 14 cm (approximately 84 l). Sufficient powdered milk was added to the water to make it opaque, and copper sulfate was added (0.125 mg/l) as an antibacterial agent.

The platforms used in the study were made of square sheets of clear plexiglass connected by a hollow plexiglass tube. Holes were drilled into the sides of the tubes to permit them to fill with water and, thus, keep them from floating. Both platforms measured 20 cm along each side. The submerged platform was 13 cm tall, and the exposed one was 15 cm tall. To equate the reward reinforcement for both platforms, the visible platform was designed to hold about 1 cm of water. The pool was divided into four quadrants, and the platforms were placed in fixed locations in the center of one of them. The water was warmed to approximately room temperature (23° to 27°C) before each experiment to eliminate thermal shock as a variable.

Dosage and Administration--Hampson et al. (1989) reproted that ranges of 0.5 to 2.0 mg/kg are effective doses to produce behavioral and hippocampal electro-physiological effects in the rat, and data accumulated in human dosage research seem to confirm this. Since we wanted to avoid hallucinations, euphoria, and changes in the rats' motor skills (Miller and Drew, 1973; Gardner et al., 1988), we selected 2 mg of Δ^9-THC/kg body weight as our dose level.

Pure Δ^9-THC was available only in gelatin capsules intended for oral use. Each contained 5 mg of Δ^9-THC dissolved in about 0.3 ml of sesame oil. This fluid was carefully extracted from the capsules and diluted with Tween to a final concentration of 1 mg of Δ^9-THC/cc. Both Δ^9-THC and placebos (pure Tween) were injected intraperitoneally approximately 1 h before testing.

Testing Procedure--Each rat was tested individually. Testing consisted of placing the rat in a large circular pool of opaque water with either an exposed or submerged platform at a fixed location within the pool. To escape the water, the rats had to find the platform and climb onto it. Rats placed into the pool with the submerged platform were required to note the position of the platform during their early trials and, in later trials, use their spatial memories to navigate to the platform. The rats placed into the pool with an exposed platform were presumably not required to use memory to find the platform, since they could see it.

Each rat was tested for 10 consecutive days, and the animals were weighed frequently throughout the 10-day period so dosage could be adjusted to accomodate their growth. On the first day, there were no platforms in the pool; the rats were simply introduced to the water and allowed to swim freely for 1 min before being removed and dried. On the following day, trials began and continued each day until day 10. During this time, each rat was given either Δ^9-THC or the placebo and was subjected to three consecutive trials per day. Testing began about 1 h after injection, since Δ^9-THC exerts maximal effect between 1 and 2 h after administration (Fehr et al., 1976; Aulakh et al., 1980; Campbell et al., 1986*b*). To eliminate diurnal variation, injections were given during the same hour each day.

Trials--A trial began when the rat was placed in the water and ended when it escaped onto the platform. The duration of each was recorded in seconds. After each escape, the rat was taken out of the pool, dried, and allowed a brief period of rest ($\geq$30 sec) before being put back into the water. On the first day, rats were allowed 5 min to find the platform, and, if they did not escape in that period, they were removed from the pool and their latency was recorded as infinity. On succeeding days, 3 min were allowed to complete each trial.

The first day's trials took so long that some experimental animals did not participate until nearly 2 h after injection. On subsequent days, however, each rat was tested within 90 min of injection. A complete group of trials, beginning with the first injection and ending with the last escape, consumed between 2 and 4 h. Trials were videotaped on days 2, 5, and 10, and the tapes were used to map the path followed by each rat to reach its goal.

Of the 43 rats that entered the testing, four rats died the second or third day, and one died on the sixth day. The data from the latter rat (a control animal) were used in statistical analysis; data for the others were discarded. A sixth rat was withdrawn from the THC-submerged platform group because it never learned how to escape. This behavior was deemed so unusual that the animal's performance was not included in statistical analysis. The final groups totalled 38 rats (9 in the invisible platform group, 10 in the control-invisible platform group, 10 in the visible platform group, and 9 in the control-visible platform group). Occasionally, there were some missing data because of experimental error, i.e., the needle came off the syringe during drug administration, spilling drug and making it impossible to verify the amount that actually was given to the rat. The latencies of escape for each rat were converted to rate (1/sec) and were computer-analyzed using an analysis of variance (ANOVA) test from the Statistical Package for the Social Sciences (Norusis, 1990). Statistical difference was determined at $P \leq 0.05$.

RESULTS

The THC-treated rats demonstrated a significantly greater improvement across trials than the untreated rats. However, this difference is almost certainly due to the fact that the THC-treated rats were generally slower on the first trial but "caught up" with the controls on subsequent trials. In general, there was no significant difference in average rate at which the rats were able to locate the platform whether it was visible or invisible. Thus, both experimental groups and both control groups were combined to examine the effect of Δ^9-THC. The experimental group, therefore, consisted of 19 rats, and the placebo group of 18 or 19 rats.

On the first trial each day, rats injected with Δ^9-THC took significantly longer time to find the platform than the controls. On the second and third trials, the experimental animals seemed to awaken to the task, and there was no significant difference between them and the control group. There was a significant improvement between trials one and three each day for both groups. A composite comparison of drug and placebo conditions for all trials 1, all trials 2, and all trials 3 clearly shows these patterns (Fig. 1). Figure 2 also shows a significant improvement in success rates between day 2 and day 10 as the rats became accustomed to the procedure.

Although there was no particular difference in testing times, there were behavioral differences noted between the drug and placebo groups. Those rats receiving Δ^9-THC were generally hyper-responsive to tactile stimuli for a few hours after drug administration. Also, a tendency of the THC-treated rats to float motionless for a while after being placed in the pool was more prominent and longer-lasting than in the control group. Analysis of the videotapes also showed that there was no real difference in distances traversed in the process of finding the platform. This means that it must have made no difference to either experimental or control rats whether the platforms were submerged or exposed.

DISCUSSION

An important aspect of this study is the lack of significant difference in the performance of control rats under different visibility conditions. The original study done by Morris (1981) showed that rats took slightly longer to navigate to the invisible platform than to the visible one. This led him to the conclusion that finding the hidden platform was the harder task. We submit that probably neither of our platforms were visible. There are several reasons. The rats that Morris (1981) used were Long-Evans rats with normal eye pigmentation and presumably normal vision. At the time our study was undertaken, Long-Evans rats were not available; therefore, we used Sprague-Dawley albinos which have restricted visual capabilities. In addition, the eyes of swimming rats are naturally quite close to the water's surface, so their range of vision is limited under the best of conditions. To further exacerbate

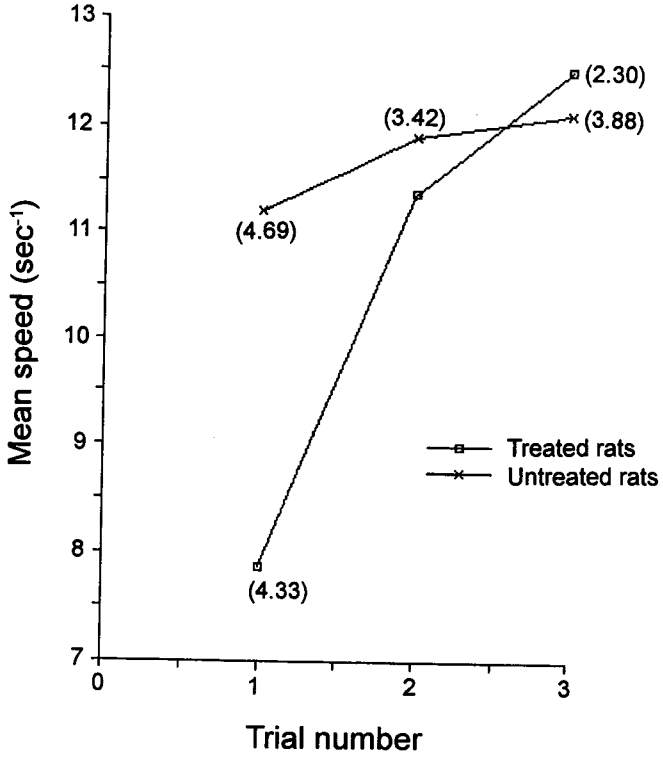

FIG. 1. Mean speeds of Δ^9-tetrahydrocannabinol-treated rats escaping from water in three trials. Standard deviations are given in parentheses.

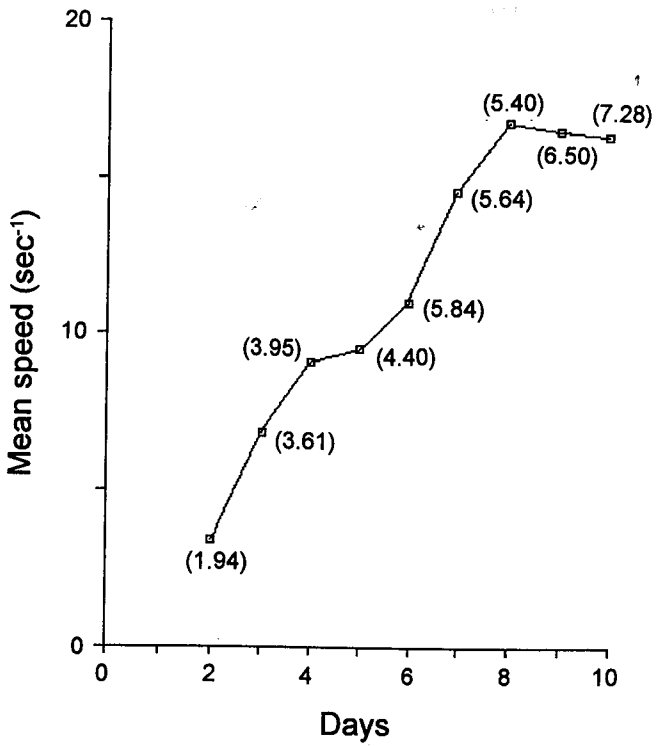

FIG. 2. Mean speeds of Δ^9-tetrahydrocannabinol-treated and untreated rats escaping from water over a 10-day period. Standard deviations are given in parentheses.

these visibility problems, Morris' (1981) visible platform was painted black, whereas ours was made of clear plastic and was filled with the milky-bluish water that surrounded it. Unfortunately, these flaws were not apparent until the experiments had been concluded and we were well into data analysis.

However, probably none of these factors affect our overall results. Whether the rats could see the platforms or not, Δ^9-THC did not affect their performance. If it had, it would have made a difference. Without a visible platform to provide a baseline, it would have been impossible to eliminate motor impairment or some other type of learning difficulty as a variable.

Despited the results of this set of experiments, we still feel that it is likely Δ^9-THC has a profound effect on spatial cognition, and we suggest that correcting the flaws and conducting more sets of experiments would be worthwhile. In experiments such as these, there is no way of montoring the effect of a drug on rats other than to note behavioral changes, and we saw none except for the initial resistance to arousal. Consequently, a suggested modification is to increase the amounts of Δ^9-THC, probably conducting several sets of trials, each with different dose levels. The levels would have to be monitored with great care. It has been reported (Domino, 1971; Weisz et al., 1982; Schulze et al., 1989) that increasing drug administration can alter responses both qualitatively and quantitatively. Also, general decreases in motor activity have been noted when doses are higher than 3 mg/kg (Miller and Drew, 1973), and, as noted previously, there is always the problem of hallucination or euphoria. Our dosages were deliberately kept low to avoid these problems, but they may have been too low to produce observable spatial-memory deficits.

The route of administration also may need reappraisal. The experiments suggesting spatial-memory defects due to marijuana have used humans as the subjects, and the drug has been administered by smoking. Smoking permits marijuana's psychoactive drugs to enter the bloodstream more rapidly and, therefore, in higher concentration than intraperitoneal injection does, and, although drug tolerances of rats and humans are very different (Fehr et al., 1976), that could be important. To further complicate things, it has been suggested that marijuana smoke may contain other psychoactive constituents besides Δ^9-THC and these other fractions may enhance the overall drug effects (Stiglick and Kalant, 1983; Schulze et al., 1989).

Even so, given the large body of evidence that suggests a definite role for Δ^9-THC in the hippocampus, particularly regarding spatial cognition, the results of this study are surprising. We agreed with Morris et al. (1982) on only one point; neither procedure seems to inhibit the learning of the non-spatial concepts that this task requires. Nevertheless, we feel very strongly that Δ^9-THC must exert a significant effect on hippocampal function. There is, after all, an entire battery of specific THC receptors present on the hippocampus, hence, some cells, probably in the brain, produce cannabinoids, and it is impossible to believe that they do not serve some specific function. Uncovering just what it is that they do in the hippocampus may help us understand the cellular mechanisms involved in learning and memory and so has tremendous potential.

The hippocampus has been identified as one brain area that is heavily predisposed to electrical seizures (Douglas, 1967) accompanied by an immediate decrease in content of acetylcholine. Such reduction in acetylcholine is also produced by Δ^9-THC administration (Essman, 1984) or electroconvulsive shock, and either can produce a persistent amnesic effect (Essman, 1986). In addition, it has been noted that Δ^9-THC increases the potency of physostigmine, a chemical that lowers

levels of acetylcholine and induces hippocampal seizures in rats (Rosenblatt et al., 1972; Gonzalez, 1985).

Clearly, the effect of cannabinoids on the hippocampus and cholinergic limbic system is profound. Thus, it becomes important to discover precisely what happens when the cannabinoid hippocampal receptors are stimulated. It will surely enhance our knowledge of hippocampal brain mechanisms and probably mechnisms in other areas as well. The full understanding of cannabinoid effects could have application in such degenerative brain diseases as Alzheimer's, Parkinson's, and Korsakoff syndrome that produce impaired memory (Miller and Branconnier, 1983) and some, like epilepsy, that can be terribly debilitating.

CONCLUSIONS

This study indicates that Δ^9-THC, at a dose of 2 mg/kg, does not impair either spatial memory or the learning that is required for navigation of a male Sprague-Dawley rat toward a specific location. Also, although Δ^9-THC was administered for 10 consecutive days, no cumulative effect of the drug was observed on these functions. In general, all four groups, regardless of drug or platform conditions, performed equally well in this task. Also, the rate of improvement was the same in both drug and placebo conditions, indicating that the ability to learn some non-spatial concepts (such as the possibility of escape and the association of escape with the mounting of a platform) is not affected by Δ^9-THC in dosages of 2 mg/kg.

The only statistically significant effect we can attribute to Δ^9-THC was that drug-treated rats took longer to "get going" on the first trial of each day. When placed in the water, drug-treated rats exhibited a prolonged (as much as 65 sec) resistance to arousal before swimming to the platform. Once started, their paths to the goal were as direct as the controls' suggesting that spatial cognition was not affected. By the second and third trials, arousal times had dropped to zero.

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