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Learning Impairment Produced in Rats by the Cannabinoid Agonist HU 210 in a Water-Maze Task

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FERRARI, F., A. OTTANI, R. VIVOLI AND D. GIULIANI. *Learning impairment produced in rats by the cannabinoid agonist HU 210 in a water-maze task.* PHARMACOL BIOCHEM BEHAV **64**(3) 555–561, 1999.—To ascertain whether the cannabinoid agonist HU 210 (25, 50, or 100 μg/kg, IP) influences rat spatial learning, water-maze performance was examined in the place (hidden platform)—and cue (visible platform)—versions of the Morris water maze. In addition, other unlearned behaviors were examined, namely, vocalization and wall hugging during the place task, and motor abilities during a motor test battery. The results obtained show that HU 210 at 50 or 100 μg/kg (once daily for 4 days, 60 min before a daily session) impaired learning in the place version but not in the cue one; wall hugging and enhanced vocalization were also displayed by the animals in the fourth session. Motor activity was compromised by the same treatment schedule. When the drug was discontinued, the effects produced by HU 210 at 50 μg/kg reversed in 3 days, while disruption of acquisition and vocalization caused by HU 210 at 100 μg/kg remained after 7 days' abstinence. Discussion centers on the possible specific cognitive mechanisms affected by the drug and on aspecific factors (i.e., anxiety-like state), which may contribute to the impairment of spatial learning. © 1999 Elsevier Science Inc.

HU 210 Cannabinoids Morris water maze Spatial learning Motor activity Vocalization Rat

CANNABINOID (CB) receptors, the molecular targets for marijuana and hashish (8), are mainly localized in brain areas (viz. hippocampus and medial-prefrontal cortex) (15,18,27) that are directly involved in the control of cognitive processes as well as motor activity (3,36,37); accordingly, Δ-tetrahydrocannabinol (THC), the main natural psychoactive CB (14), appears to affect certain aspects of these functions both in human and in laboratory animals (1,19). Several experimental studies are consistent with a hippocampal mediation of the disruptive effects of CBs on learning and memory (23). CB agonists have been found to inhibit hippocampal and medial-prefrontal cortex long-term potentiation (33), a synaptic change suggested as a neural mechanism for information storage in the brain (21,40); moreover, most of these drugs reduce choline uptake and brain acetylcholine levels (24,30) and impair memory tasks.

However, not all CBs seems to have a negative effect on learning and memory (22,23). What is more, as CBs also lead to a broad range of motoric, emotional, and motivational changes (29), it is important to assess the involvement of these factors in the effects attributed to memory and learning deficits.

The purpose of the present study was to examine the influence of the potent synthetic CB agonist HU 210 (20,25) on the acquisition and retrieval of reference memory in the Morris water maze (31), while taking into account other unlearned behaviors typically modified by CBs, namely, vocalization (17) and motor activity (6). To this end, different experiments were performed using 1) water-maze place version, 2) water-maze cue version, and 3) motor test battery.

GENERAL METHOD

Animals

The subjects were male outbred Wistar Hannover rats (Harlan Nossan, Udine, Italy) weighing 230–250 g at the outset. They were housed in groups of four, in standard Macro-

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lon cages (57 × 35 × 19 cm), with food and water ad lib, and on a 12-h light cycle, from 0700 to 1900 h, for at least 1 week prior to the start of the experiments.

The regulations in force on the care of animals for scientific purposes (CEE Council 86/609, Italian D.L. 27/01/92 No. 116) were strictly complied with.

Morris Water Maze: Apparatus

The circular water maze tank (130 cm in diameter, 60 cm in depth) was constructed of white Plexiglas. Prior to testing, the tank was filled to a depth of 35 cm with water maintained at 30 ± 2°C and made opaque by the addition of whole milk. A white cylindrical escape platform (10 cm in diameter) remained submerged 1 cm below water level, whereas a black platform of the same dimension protruded 1 cm above the surface of the water. Only one platform was placed in the tank during any phase of testing. The white and black platforms, respectively, were used in the place (Experiment 1) and cue (Experiment 2) versions of the Morris task (31). The tank was virtually divided into four quadrants by two wires intersecting at right angles. The many extramaze cues were kept approximately 1 m from the tank, and comprised not only sundry standard laboratory items but also the observers themselves, who were unaware of the test protocol. Following each day of testing the tank was drained and cleaned.

Motor Test Battery

The motor test battery used was modified from that described by Bjorklund et al. (4) to evaluate only motor orientation and coordinated limb use on each side of the body; the response in each test was rated on a three-point scale. Initially, each animal was observed unrestrained on the bench for 2 min to ascertain general posture asymmetry, as inclination, more or less marked, of the head (0 = absent, 1 = weak, and 2 = strong), hypokinesia, as animal's exploring behavior (0 = normal exploration, 1 = reduced exploration, 2 = no exploration) and spontaneous rotation, as rotatory movement of at least 180° (0 = no rotation; 1 = one to three rotations; 2 = more than three rotations). Subsequently, limb reflexes and coordinated limb use were assessed.

Forelimb placement. The rat was grasped around the abdomen and slowly head first towards the surface of the bench, any inaccuracy or lack of coordination in the reflex placement of the forelimbs being rated on a scale 0–2, where 0 = symmetrical landing, 1 = the animal lands with only one paw, 2 = asymmetrical landing involving the body.

Forelimb suspension. Using thumb and forefinger, the experimenter held the rat up by one forepaw and the speed with which it grasped the experimenter's finger with its free paw to pull itself up onto the experimenter's hand was rated on the scale 0–2, during a 5-s observation period, where 0 = immediate response, 1 = slow response (1–5 s), 2 = no response. The test was then repeated with the other forepaw. In a further test (5-s observation period), the rat was left hanging from a wooden bar and the inability with which it pulled itself up was rated as follows: 0 = the animal rises, 1 = the animal hangs up, 2 = the animal falls down.

Climbing grid. The rat was placed on a vertical wire grid with horizontal grills (a removable cage floor clamped vertically by its upper edge to the bench surface) and its climbing inability rated on the same scale as before.

Pyramidal signs. The rat was placed at the edge of the bench, with an hindpaw hanging on to the vertical grid; the speed with which it retracted its paw was rated, during a 5-s observation period, as follows: 0 = rapid retraction (within 1 s); 1 = slow retraction (more than 1 s), 2 = no retraction.

Catalepsy. The animal was placed sitting on its hindpaws and its ability (scored 0) or inability (scored 1) to resume a four-paw posture was evaluated.

At the end of the test battery, a cumulative score (as sum of the rating scores across tests) was given to each treatment group.

Drugs and Treatments

HU 210 (Tocris Cookson, Bristol, UK) was freshly prepared, being dissolved in vehicle [suspension containing a drop (0.1%) of Tween 80 and distilled water] at concentrations that allowed the administration of 1 ml/kg. The doses used were chosen on the basis of previous experiments (11). HU 210 was intraperitoneally (IP) administered.

EXPERIMENT 1: PLACE VERSION

The effect of HU 210 on spatial learning was assessed by testing the influence of the drug on the acquisition of the task by naive animals.

Method

In the place version of the water maze, the submerged platform was located in one of the four quadrants of the tank, and remained there throughout training. Thirty-two rats were randomly divided into four groups (n = 8). The animals received IP injections of HU 210 at 25, 50, or 100 μg/kg or vehicle (once daily for 4 days) and were trained on four consecutive daily sessions in the place navigation task. The daily session started 60 min after each treatment. Each session consisted of four trials; in each trial the subject was placed in the water, facing the edge of the tank, in one of four start locations. The order of the start locations was varied in a random fashion. If the subject did not locate the platform within 60 s it was gently guided there by the experimenter and allowed to remain for 20 s, as were the animals that found the platform by themselves. After the fourth session, all treatments were discontinued; the animals were subsequently subjected to sessions at 24 h, 3, and 7 days after the last treatment (abstinence) (fifth, sixth, and seventh session, respectively).

In each session, the following parameters were evaluated for each treatment group as the mean of four trials: escape latency (max: 60 s): mean of the total time to reach the platform; time spent outside the target quadrant (max: 60 s): mean of the total time spent by the animal in the three quadrants of the pool that did not contain the platform.

Vocalization is reported for each treatment group as total number of vocalizations displayed by the animals when removed from the platform.

In the first and fourth session, the strategy employed by the animal to reach the platform was also observed and every episode of wall hugging (defined as a complete turn of the pool in contact with the wall) was noted. This parameter is presented as the number of animals displaying wall hugging per session.

Results

Data obtained for escape latency (Fig. 1) were analyzed using the General Linear Model procedure (GLM) (four treatment groups by 7 days by seven blocks). A significant effect of HU 210 was obtained for treatments, $F(3, 28) = 7.8$, $p = 0.001$, for days, $F(6, 168) = 37.4$, $p = 0.000$, and for days ×

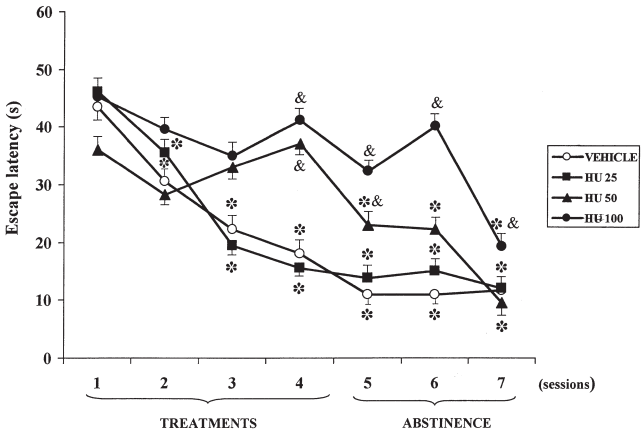

FIG. 1. Effect of HU 210 on escape latency in Morris water maze place version. HU 210 (HU) or vehicle were administered 60 min before the first four sessions; 5, 6, and 7 represent the sessions performed after 24 h, 3 days' and 7 days' drug suspension (abstinence), respectively. Each point is the mean ± SEM of the values for each treatment group. &Significantly different from vehicle-treated rats in the same session (ANOVA followed by Student–Newman–Keuls' test); *significantly different from the same animal treatment group in the first session (ANOVA for repeated measures followed by Student–Newman–Keuls' test).

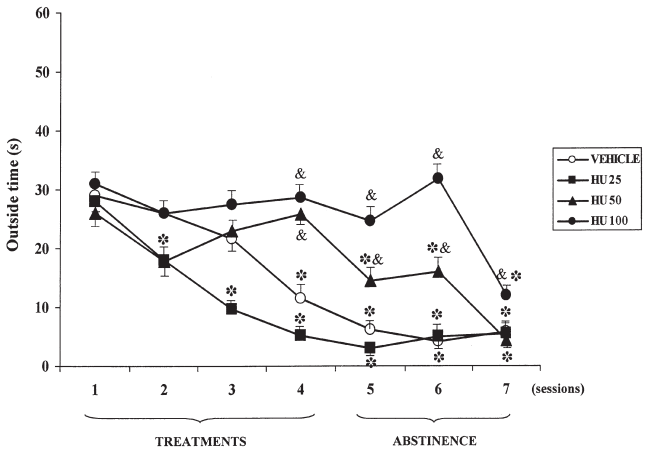

FIG. 2. Effect of HU 210 on time spent outside the target quadrant in Morris water maze place version. Legend as in Fig. 1.

treatments, $F(18, 168) = 3.5$, $p = 0.000$. As shown in Fig. 1, controls, as well as rats injected with HU 210 at 25 μg/kg, displayed a time-dependent decrease in escape latency over seven sessions [ANOVA for repeated measures: $F(6, 42) = 17.7$, $p = 0.000$; $F(6, 42) = 33.3$, $p = 0.000$, respectively], and acquisition was already apparent in the second session. Rats treated with 50 and 100 μg/kg of HU 210 did not show any improvement during the first four sessions, so that by the fourth one their escape latencies were significantly higher than those of the controls [ANOVA: $F(3, 28) = 11$, $p = 0.000$]. Twenty-four hours, 3, and 7 days after the last injection (fifth, sixth, and seventh session, respectively) rats treated with 50 μg/kg time dependently improved their performance to achieve control values by the seventh session [ANOVA for repeated measures: $F(6, 42) = 12.9$, $p = 0.000$]. Despite the interruption of treatment, rats injected with 100 μg/kg of HU 210 did not exhibit any behavioral modification until the seventh session, when they improved their performance with respect to the previous sessions [ANOVA for repeated measures: $F(6, 42) = 4.1$, $p = 0.003$]. Their escape latency remained significantly higher than that of the other three groups in the fifth [ANOVA: $F(3, 28) = 10.8$, $p = 0.000$], sixth [ANOVA: $F(3, 28) = 14.7$, $p = 0.000$], and seventh [ANOVA: $F(3, 28) = 4$, $p = 0.02$] session. In view of the long-lasting effects displayed by HU 210 at 100 μg/kg, a further test was performed at a 15-day abstinence only for this group: escape latency was still high with respect to controls (vehicle = 11 ± 1.5, HU 100 μg/kg = 18 ± 0.4; $t = 4.5$, $p = 0.000$, Student's t-test) (data not reported in Fig. 1).

Data obtained for the time spent by the animals outside the target quadrant (Fig. 2) were preliminarly analyzed using GLM (four treatment groups by 7 days by seven blocks). A significant effect of HU 210 was obtained for treatments, $F(3, 28) = 6.1$, $p = 0.002$, for days, $F(6, 168) = 20.2$, $p = 0.000$, and for days × treatments, $F(18, 168) = 3.2$, $p = 0.000$. In the controls, the time spent outside the target quadrant was found to be significantly shorter from the fourth session onwards [ANOVA for repeated measures: $F(6, 42) = 9.6$, $p = 0.000$]; in the animals treated with the lowest dose the improvement yet appeared in the second session [ANOVA for repeated measures: $F(6, 42) = 19.4$, $p = 0.000$] and in those treated with 50 μg/kg during abstinence (fifth, sixth, and seventh session) [ANOVA for repeated measures: $F(6, 42) = 8.5$, $p = 0.000$]. Finally, in the rats treated with the highest dose the time decreased only in the seventh session [ANOVA for repeated measures: $F(6, 42) = 3.01$, $p = 0.01$].

During the water-maze test, the animals of the various treatment groups differed for their vocalization (Fig. 3) [($K = 7$, $n = 4$) $\chi^2_r = 18.9$, $p < 0.05$; Friedman's test]. Rats injected with HU 210 at 50 and 100 μg/kg vocalized strongly during the first five and six sessions, respectively; this enhanced behavior gradually and dose dependently diminished during the last sessions.

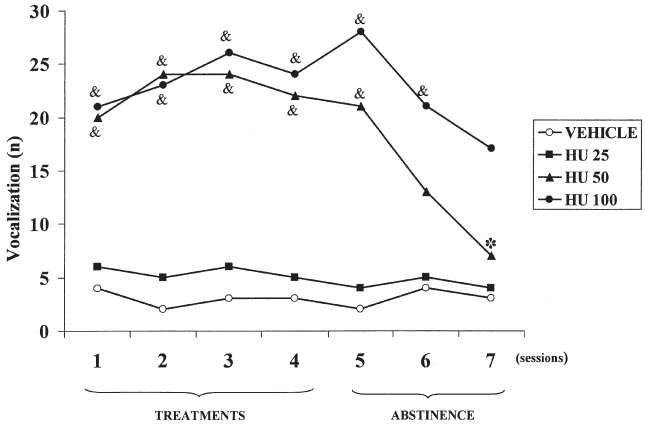

FIG. 3. Effect of HU 210 on vocalization in Morris water maze place version. HU 210 (HU) or vehicle were administered 60 min before the first four sessions; 5, 6, and 7 represent the sessions performed after 24 h, 3 days' and 7 days' drug suspension (abstinence), respectively. Each point represents the total number of vocalizations for each treatment group significance as in Fig. 6.

In the first session all treatment groups behaved similarly in their search strategy, and the number of animals displaying wall hugging for each group was not statistically different (vehicle: 3/8; HU 25:2/8; HU 50:4/8; HU 100: 3/8) (data not graphically presented). However, in the fourth session, rats treated with 50 and 100 μg/kg of HU 210, followed a thigmotactic search pattern, unlike the controls, which at this point adopted a direct search [vehicle: 0/8; HU 25: 0/8; HU 50: 3/8 ($\chi^2 = 6.8, p = 0.03$) (2×3); HU 100: 6/8 ($\chi^2 = 15.3, p = 0.002$) (2×4)].

EXPERIMENT 2: CUE VERSION

The HU 210-induced impairment of the acquisition of the place navigation task, observed in Experiment 1, could reflect not only interference with place learning but also drug related swimming deficits. To assess this possibility, the effect of HU 210 on the capability of rats to escape to a visible platform was tested.

Method

In the cue version of the water maze, the test room, the tank, and the procedure were identical to those used for the place version, except that the submerged platform was replaced by a raised one in the same quadrant of the tank. Twenty-four experimentally naive rats were randomly divided into four groups ($n = 6$) that received IP injections of HU 210 at 25, 50, 100 μg/kg or vehicle once daily for 4 days; a daily session started 60 min after the treatments. After the fourth session, all treatments were discontinued; the animals were subsequently submitted to a daily session at 24 h and 3 days during abstinence (fifth and sixth session, respectively). In each session, escape latency and time spent outside the target quadrant were evaluated.

Results

Data obtained for the escape latencies (Fig. 4) were preliminarly analyzed using GLM (four treatment groups by 6 days by six blocks). No significant difference was obtained for treatments, $F(3, 20) = 1.0, p = 0.4$, and for days × treatments, $F(15, 100) = 1.1, p = 0.3$, while a significant modification was obtained for days, $F(5, 100) = 172.3, p = 0.001$. As shown in Fig. 4, controls submitted to the sessions time dependently reduced their escape latency, which remained constant during the fifth and sixth sessions [ANOVA for repeated measures: $F(5, 30) = 56, p = 0.000$]. Animals treated at the various doses of HU 210 (25, 50, and 100 μg/kg) behaved similarly [ANOVA for repeated measures: $F(5, 30) = 24, p = 0.000$; $F(5, 30) = 165, p = 0.000$; $F(5, 30) = 24, p = 0.000$, respectively]. These results were confirmed by the time spent outside the target quadrant (Fig. 5); in fact, GLM procedure showed a significant difference only for days, $F(3, 100) = 95.3$, $p = 0.000$.

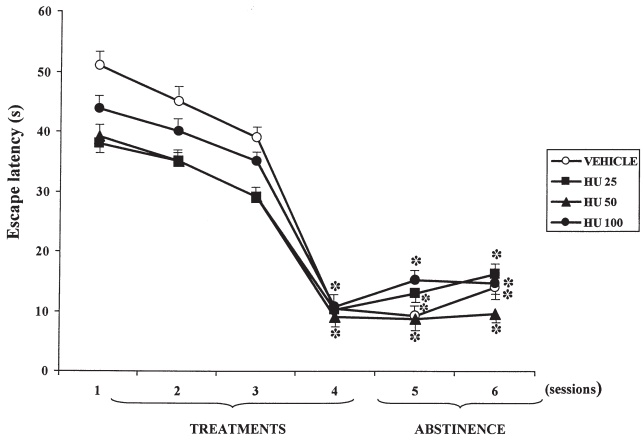

FIG. 4. Effect of HU 210 on escape latency in Morris water maze cue version. HU 210 (HU) or vehicle were administered 60 min before the first four sessions; 5 and 6 represent the sessions performed after 24 h and 3 days' drug suspension (abstinence), respectively. Each point is the mean ± SEM of the values for each treatment group. *Significantly different from the same animal treatment group in the first session (ANOVA for repeated measures followed by Student–Newman–Keuls' test).

EXPERIMENT 3: MOTOR TEST BATTERY

To assess the effects of HU 210 on general motor activity, naive animals were submitted to a motor test battery.

Method

Twenty-four rats were randomly divided into four groups ($n = 6$) that received IP injections of HU 210 at 25, 50, 100 μg/kg or vehicle once daily for 4 days. A daily motor test was performed 60 min after the treatments on the first and the fourth day (first and second test). Subsequently, all treatments were discontinued, and the animals were submitted to a test at 24 h, 3, and 7 days during abstinence (third, fourth, and fifth test, respectively).

Results

Motor activity scores (Fig. 6) were analyzed using Friedman's test, which showed significant differences for days × treatments [(K = 5, $n = 4$) $\chi^2_r = 12.7, p < 0.05$].

Treatments with the lowest dose never modified the animals' behavior with respect to controls. A Mann–Withney *U*-test showed a significant impairment of motor abilities after 50 and 100 μg/kg of HU 210, whether acutely ($t = 55$ and $t = 57$,

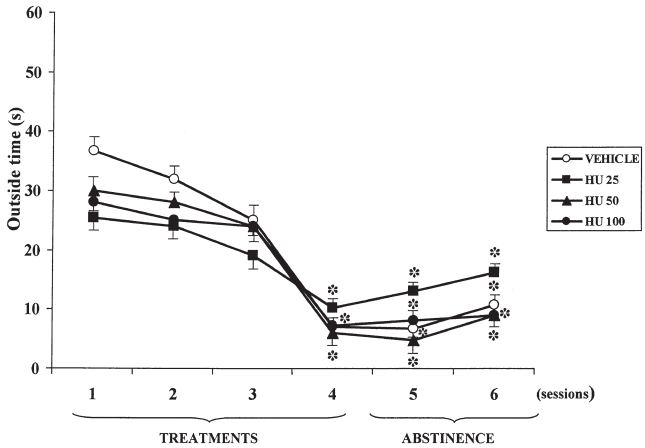

FIG. 5. Effect of HU 210 on time spent outside the target quadrant in Morris water maze cue version. Legend as in Fig. 4.

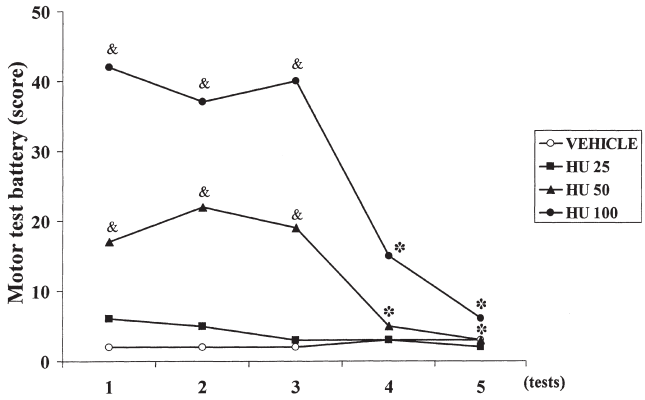

FIG. 6. Effect of HU 210 on motor test battery. HU 210 (HU) or vehicle were administered 60 min before the first and the second test (corresponding to acute or four injections, respectively); 3, 4, and 5 represent the tests performed after 24 h, 3 days' and 7 days' drug suspension (abstinence), respectively. Each point represents the cumulative score for each treatment group. &Significantly different from vehicle-treated rats in the same session and *significantly different from the same animal treatment group in the first session (Kruskal–Wallis followed by Mann–Withney *U*-test).

respectively; $p < 0.05$) (first test) or subchronically administered ($t = 57$ for both; $p < 0.05$) (second test), and this effect was still visible 24 h after drug suspension ($t = 53$ and $t = 57$, respectively; $p < 0.05$) (third test).

All the data obtained in this study are summarized in Table 1.

DISCUSSION

The hippocampus is known to play an essential role in cognitive processes. Given the high hippocampal concentration of CB receptors (18,27) and the neurochemical and electrophysiological changes that occur in this area after CB treatment (15,19,39) a disruptive influence of HU 210 on learning and memory would be predictable. However, the investigation of the influence of CBs on cognitive abilities has led to controversial results in both human and laboratory animal research. Systemic administration of THC, as well as of the novel CB agonists WIN55,212-2 and CP 55,949, have been seen to impair learning and memory in rodents; on the other hand, neither anandamide, the putative endogenous CB ligand (13), nor cannabidiol, a naturally occurring CB, have any apparent effect on memory tasks (23).

The results obtained in the present study show that HU 210, acutely or subchronically administered at 25 μg/kg, did not negatively modify rat behavior, while at higher doses (50 and 100 μg/kg), it provoked several important effects, among which was learning disruption. These behavioral modifications were dose and time related: all gradually disappeared within 3 days from the suspension of subchronic 50 μg/kg, but, in the case of subchronic 100 μg/kg, some of them, namely vocalization and impaired spatial learning, were present even during the last session, after 7 days' abstinence.

As already described for HU 210 and other CBs (6,11,35), the animals' motor activity was found to be compromised by the drug at 50 and 100 μg/kg. The rats treated with these two doses, despite a state of marked sedation, were hypersensitive to tactile stimuli and vocalized strongly when touched. Vocalization is considered a pointer of cannabimimetic activity (17), and it is elicited by THC at doses much higher than those of HU 210. This sign seems to reflect heightened emotionality associated with a state of fear, and the same anthropomorphic interpretation has been made with regard to aggressive reactions often observed after CBs (7,29), in accordance with dysphoria, anxiety, and panic, which have been described in humans after high doses of marijuana and hashish (12,42). The correlation between CBs and stress has long been proposed (26) and supported by experimental findings on animals, where CBs induce a potent secretion of adrenocorticotropin hormone (ACTH) (9) and corticotropin releasing factor (CRF) (7), which, as is known, play a key role in stress (10,16). Moreover, the attenuation exerted by the CRF antagonist D-phenyl CRF12-41 on rat anxiogenic responses to HU 210 (7) strongly suggests the mediation of endogenous CRF systems in these effects. In previous studies, we verified that HU 210, when subchronically administered at 50 and 100 μg/kg, besides potentiating novelty-induced grooming, behaved as an anxiogenic in the x-maze test (data not yet published), and these behavioral patterns are also accepted as pointers of modified emotionality and/or a state of fear.

All in all, our results would indicate that drug-reduced motor activity is not responsible for learning impairment in the place version of the Morris water maze, for rat performance in the cue version was similar in all treatment groups and in the various sessions. The results obtained in Experiment 2 also suggest that HU 210–treated rats, besides having a sufficient motor ability to reach the visible platform, were moti-

TABLE 1

A SUMMARY OF THE EFFECTS PRODUCED BY HU 210 IN THE VARIOUS EXPERIMENTS

Treatment (μg/kg)	Place Version Performance			Vocalization			Wall Hugging		Cue Version Performance			Motor Test Battery Performance		
	Ac	S-chr	Abst	Ac	S-chr	Abst	Ac	S-chr	Ac	S-chr	Abst	Ac	S-chr	Abst
HU 25	—	↑	—	—	—	—	—	—	—	—	—	—	—	—
HU 50	—	↓	—	↑	↑	—	—	—	—	—	—	↓	↓	—
HU 100	—	↓	↓	↑	↑	↑	—	↑	—	—	—	↓	↓	—

HU = HU 210; Ac = acute treatment; S-chr = subchronic treatment; Abst = 7 days' abstinence.

↓, ↑, or — indicate significant decrease, increase, or no modification of the behavior, respectively, in relation to appropriate vehicle-treated animals.

vated to perform the task. In fact, it was noted, particularly after 100 μg/kg, that the animals swam normally, apart from a few seconds initial immobility; moreover, when some of them lost their balance on the platform and fell into the water, they managed, with some difficulty, to climb up again. While, therefore, motor deficit cannot justify the prolonged escape latency, it is likely that heightened emotionality produced by the drug at high doses may contribute to the disruptive effects on spatial learning. It is well established that stress and arousal affect rats' performance, and high corticosterone and ACTH levels, as measures of stress response, have been found to be correlated with a deterioration of learning in memory tasks (2,5,37,38). In our experiments, analysis of the time spent outside the target quadrant and of the directions in which the animals swam in the fourth session indicates that rats subchronically treated with HU 210 at 50 and 100 μg/kg not only did not know the exact position of the platform but persisted in trying to escape along the walls of the pool. Wall hugging has been observed in hippocampectomized rats (37), but it also suggests an anxietylike state (32), for it is displayed to a certain degree in some controls only during the first sessions.

It is to pointed out that HU 210 at 25 μg/kg slightly facilitated place learning. Quite possibly, low stress was beneficial to performing the task, because, as already suggested (28) ". . . optimal level of anxiety is required for place learning with shifts in either direction resulting in suboptimal performance."

As it has been reported that HU 210 induces hypothermia in rats in a dose-dependent manner (34), it cannot be excluded that a related discomfort might contribute to the anxiety-like state of animals, resulting in an inefficient learning after high doses of HU 210. However, a significant hypothermia was obtained after an hour from the intracerebral injection of the compound (34), and our preliminar experiments showed that, 1 h after IP injection of HU 210 at 50 and 100 μg/kg, the rat rectal temperature was lowered of about 1°C only (data not reported).

Once subchronic treatment at 50 μg/kg was discontinued, the animals very rapidly learned the position of the platform, for which there are two possible explanations: 1) HU 210 produces temporary and partial damage to specific cognitive mechanisms in the hippocampus; 2) the drug, at least at this dose, does not disrupt acquisition or retrieval, but rather interferes with their expression, owing to the abnormal level of anxiety. In any case, the effect on cognition was reversible within a few days. On the other hand, the possibility of a more specific and permanent impairment of learning cannot be excluded in the case of the highest dose, for in Experiment 1, even 15 days after the suspension of treatment, the animals still appeared disoriented, despite the numerous sessions performed. It is not surprising that the anxiety-like state elicited by CBs could affect the rats' performance in the place version and not in the cue version of the water task, because, as already pointed out, the two tests involve different degrees of stress (41).

In conclusion, our work confirms that HU 210 shares with other CBs the ability to interfere with learning processes: this finding is consistent with the neurochemical and electrophysiological hippocampal changes produced by these drugs. However, it remains to be seen whether the effect is primarily due to a direct activity of the drug on receptor-mediated cognitive mechanisms or, rather, is the result of a highly charged emotional state brought on by the drug.

ACKNOWLEDGEMENTS

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