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Spontaneous Behavior and Sleep-Wakefulness Cycle in Isolated and Paired REM Sleep Deprived-Marihuana Treated Rats[1]

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MONTI, J. M. AND E. A. CARLINI. *Spontaneous behavior and sleep-wakefulness cycle in isolated and paired REM sleep deprived-marihuana treated rats.* PHARMAC. BIOCHEM. BEHAV. 3(6) 1025–1029, 1975. – The correlation between marihuana-induced aggressive behavior and changes in the sleep-wakefulness cycle was studied in chronically implanted rats. Marihuana injection in non-REM deprived rats did not induce aggressiveness irrespective of the animals being caged in isolation or paired. During this procedure quantization of the sleep-awake cycle revealed that wakefulness was increased while slow wave and REM sleep were decreased, mainly in the paired animals. REM deprived-marihuana injected animals recorded in isolation behaved like the control solution-injected rats. They showed a large rebound of REM and were not aggressive at the end of the 8 hr sessions. Conversely, when these animals were paired during the recording periods, they remained continuously awake and showed numerous episodes of aggressiveness. These results suggest that the aggressiveness inducing properties of marihuana are related to the REM deprivation and to the increased environmental stimulation achieved by pairing the animals.

Marihuana Aggressive behavior REM sleep Sleep-wakefulness cycle

IT has been shown that rats previously deprived of REM sleep, injected with cannabis and paired, display an irritable and aggressive behavior characterized by boxing posture, vocalization and biting of their cagemates and inanimate objects [1,2].

Electroencephalographic effects of acute administration of cannabis to rats have also been described [8, 9, 10], with the most noticeable changes being the reduction of EEG voltage and the appearance of spike discharges during wakefulness and the REM phases. Similar effects have been observed also in rabbits [7] and monkeys [5].

The following experiments were carried out to analyze the effects of marihuana on the sleep-wakefulness cycle of REM deprived and control rats, and to determine whether alterations of the rat's environment would influence the changes induced by marihuana. As marihuana-aggressiveness following REM deprivation was observed only after pairing the rats [1], special attention was given to the condition of the recording sessions. Thus, recording sessions were run with paired and individually caged animals.

METHOD

Animals

Seventeen male Wistar rats weighing 250–300 g were used for the EEG studies. They were housed individually and maintained under controlled environmental conditions with a cycle of 12 hr light–12 hr darkness. All the animals were anesthetized with sodium pentobarbital (40 mg/kg) and chronically implanted with bipolar Nichrome electrodes (200 μ dia.) in the frontal and occipital cortices, dorsal hippocampus and neck muscles. All the electrodes were wired to a small connector fixed to the skull with dental acrylic.

Procedure

Ten days after implantation, each animal was placed in a sound-proof box and sleep patterns were recorded using a Beckman polygraph. When the animals were fully adapted to their new environment as judged by the consistency of their sleep-wakefulness cycles, experiments were started. All records were scored for waking (W), slow wave (SWS) and REM sleep (REM), according to standard criteria [6].

Recordings from isolated rats. Seven rats were used in this phase. EEG recordings were obtained in a random order from each animal after the following treatments: (A) IP injection of 1 ml/kg of 0.7 percent Tween 80-saline (control solution); (B) IP injection of 10 mg/kg of a marihuana extract containing 6.85 mg of Δ^9-tetrahydrocannabinol (THC) and 0.54 mg of cannabinol; (C) 4 days of REM deprivation followed by IP injection of

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control solution, and (D) 4 days of REM deprivation followed by IP injection of 10 mg/kg of the marihuana extract. REM deprivation was achieved through the flower pot technique as routinely employed by us [1,2] and 7 to 10 days elapsed between each 2 treatments. All recording sessions, lasting 8 hr, started 20 min after the injection.

Recordings from paired rats. These were made in a random order, after the following treatments: (E) 4 days of REM deprivation followed by IP injection of control solution; (F) IP injection of 10 mg/kg of the cannabis extract, and (G) 4 days of REM deprivation followed by 10 mg/kg of the cannabis extract. Twenty min after the injections 2 similarly treated rats were connected to the recording wires and introduced together into a recording cage measuring 30 x 40 x 30 cm. Recording sessions lasted 8 hr and started 20 min after injections. At least one week elapsed between experiments.

Results were analyzed by analysis of variance for dependent samples, followed by multiple comparisons using the Scheffé test [12].

In order to determine if the EEG recording procedure could affect the aggressive behavior induced by marihuana in the REM-deprived animals, 6 additional rats were implanted as above, except that indwelling electrodes were not attached to a connector. Thereafter, they were REM deprived, injected with 10 mg/kg of the marihuana extract and paired. Aggressive behavior of these pairs was scored for 8 hr, since that was the time the animals remained in a mutual upright position, as previously described by us [1,2].

RESULTS

Isolated rats

Cannabis induced the appearance of intermittent bursts of high-voltage polyspikes during wakefulness and REM. In the REM deprived animals, the polyspikes frequently overrode the cortical EEG during REM. However, the presence of a tonic hippocampal theta rhythm and the absence of neck muscle activity permited the recognition of REM episodes (Fig. 1). The amount of polyspikes per min between the nondeprived marihuana injected (Treatment B, see method) and the REM deprived-marihuana treated animals (treatment D) did not differ significantly (Student *t* test).

Cannabis, in nondeprived and individually recorded rats, significantly increased the latency for the appearance of the first REM period (REMP) and decreased the number of

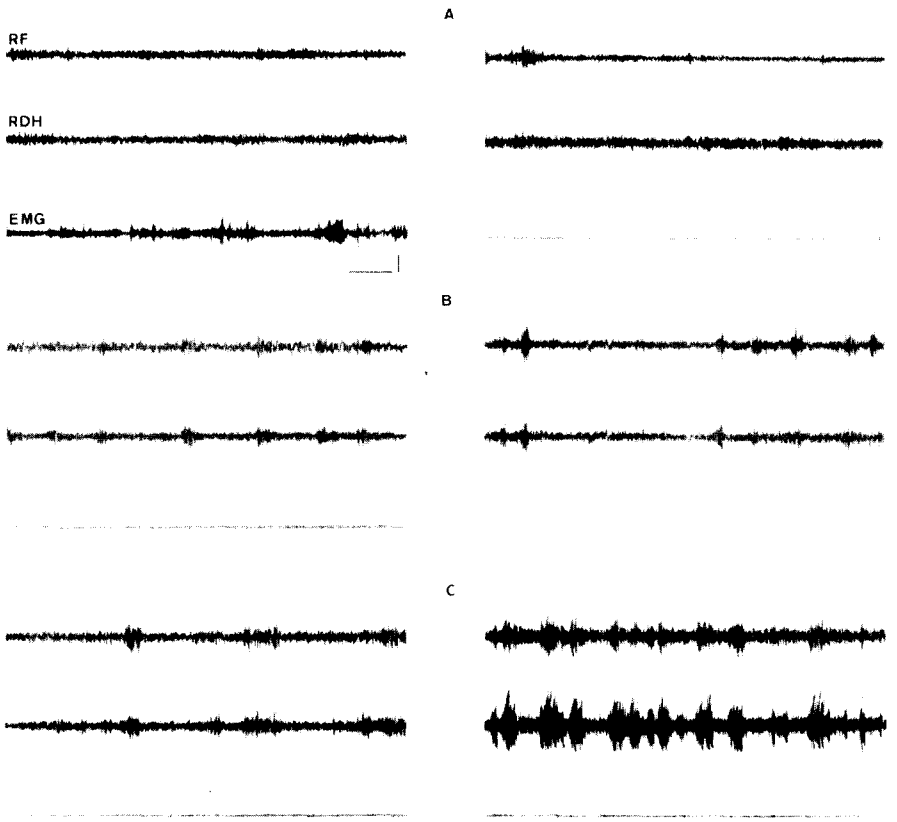

FIG. 1. EEG activity during wakefulness and REM sleep in nondeprived and REM sleep deprived animals after the injection of 10 mg/kg IP of a marihuana extract. Abbreviations: RF right frontal cortex; RDH right dorsal hippocampus; EMG neck muscles activity; scale 10 sec, 100 μV. Left and right columns correspond to records obtained during wakefulness and REM sleep respectively. (A) saline-tween 80 (control solution) injection. (B) cannabis extract (10 mg/kg IP) injection. (C) cannabis extract injection in a REM sleep deprived animal. The marihuana extract induced the appearance of intermittent bursts of high voltage polyspikes during wakefulness and REM. In the deprived animals they almost totally overrode the EEG during REM sleep.

TABLE 1

EFFECTS OF 10 MG/KG OF CANNABIS EXTRACT ON SOME VARIABLES OF THE SLEEP-WAKEFULNESS CYCLE OF NORMAL AND REM DEPRIVED RATS

Treatment	Rat Conditions: REM Deprivation	Rat Conditions: Recording	Drug	Latency to 1st REM Period (REMP) (min ± SE)	No. of REMP (± SE)	Mean Duration of REMP (sec ± SE)
A	none	individual	control solution	49 ± 8	21 ± 0.5	186 ± 10
B	none	individual	cannabis	149 ± 24†	15 ± 2*	199 ± 19
C	4 days	individual	control solution	24 ± 7	27 ± 2*	412 ± 55†
D	4 days	individual	cannabis	28 ± 10	27 ± 2*	304 ± 25†
E	4 days	paired	control solution	28 ± 11	35 ± 6*	175 ± 15
F	none	paired	cannabis	207 ± 48†	14 ± 3	89 ± 16
G	4 days	paired	cannabis	no REMP were present		

* and † differ significantly from control solution-treated individually recorded rats: *$p<0.05$ †$p<0.01$

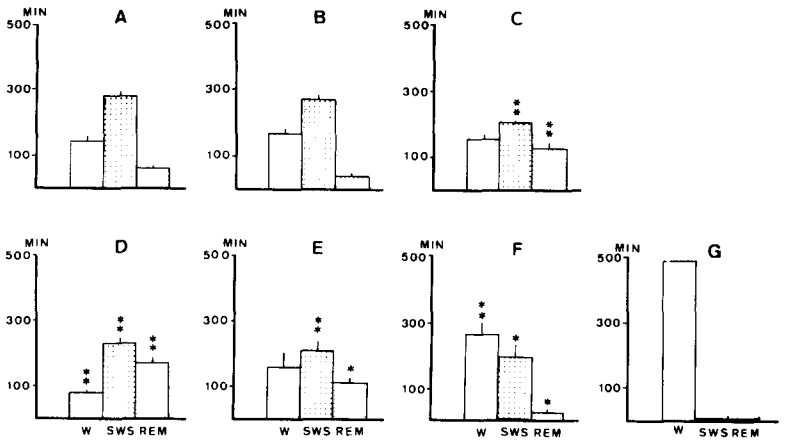

FIG. 2. Amounts of time spent in wakefulness (W, open columns), slow wave sleep (SWS, columns with squares) and REM sleep (REM, dotted columns) during 6 different experimental situations. Recordings from animals: (A) isolated and injected with control solution (0.7 percent Tween-80-saline); (B) isolated and injected with 10 mg/kg, IP of a marihuana extract; (C) REM deprived (96 hr), isolated and injected with control solution; (D) REM deprived, isolated and injected with a marihuana extract; (E) REM deprived, paired and injected with control solution; (F) paired and injected with a marihuana extract; (G) REM deprived, paired and injected with a marihuana extract. Bars represent mean values ± s.e. of 8 hr sessions. *$p<0.05$ **$p<0.01$.

REMP (Table 1, Treatment B). Waking time increased and REM diminished although without attaining significance (Part B of Fig. 2). In these animals, REM distribution was substantially modified by the drug, being absent during the first hour and much decreased during the second one (Part B of Fig. 3).

REM deprived animals having either an injection of control solution or marihuana and kept isolated during the recording sessions showed both a large rebound of REM together with a higher number of REMP and a short latency

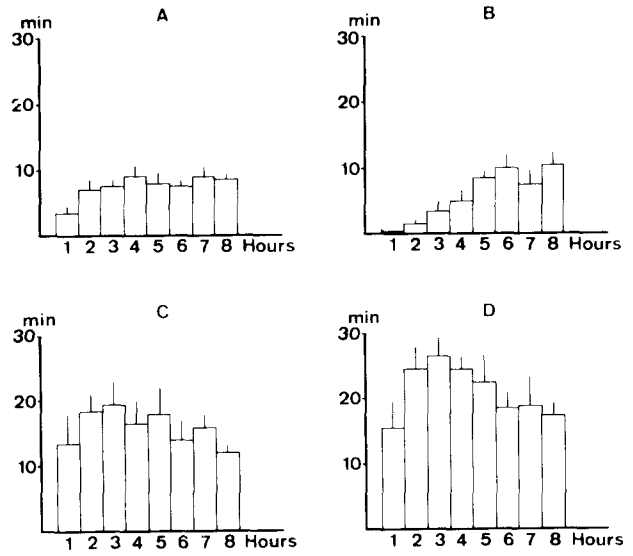

FIG. 3. The effect of 10 mg/kg of a marihuana extract on REM sleep distribution during 8 hr sessions. All experiments correspond to animals recorded in isolation. (A) control solution injected; (B) marihuana extract injected; (C) REM deprived (96 hr) and control solution injected; (D) REM deprived and marihuana extract injected. Bars represent the average amount of REM sleep ± s.e. during each hour of the recording session.

for the appearance of the first episode (Parts C and D of Figs. 2 and 3; Table 1, Treatments C and D). They also showed an increase of SWS (Fig. 2). At the end of the sessions both groups of animals were easily handled by the experimenters.

Paired rats

When 2 REM deprived-control injected animals were paired (Table 1 and Fig. 2, treatment E), sleep started after an initial period of 32 ± 3 min, spent mainly in grooming. Thereafter, their sleep-awake cycle was similar to that observed when they were in isolation (Table 1 and Fig. 2, Treatment C). In contrast, when 2 REM deprived rats were injected with marihuana and paired during the recording session (Table 1 and Fig. 2, Treatment G), the EEG revealed that they remained continuously awake with episodes of aggressiveness and short periods of synchronized EEG although behaviorally aroused. The time spent in fighting during the recording period (5.8 ± 2.5 hr) did not differ significantly (Student *t* test) from that observed in the implanted but not recorded animals (6.2 ± 2.3 hr). Handled after the 8 hr session those animals reacted viciously, biting or trying to escape from the experimenter. Paired, marihuana injected but non-REM deprived animals (Table 1 and Fig. 2, Treatment F) did not show aggressiveness. Quantization of their sleep-awake cycles evidenced a significant decrease of SWS and REM.

DISCUSSION

Confirming previous findings [8, 9, 10], it was shown that marihuana induced the appearance of polyspikes in the cortical EEG during wakefulness and REM. Although REM was decreased after marihuana in the nondeprived animals, the compound did not prevent the REM-rebound in the deprived ones, when they were recorded in isolation.

There were striking differences in the spontaneous behavior and sleep-awake cycles of the REM deprived-marihuana injected animals when comparisons were done between preparations recorded in isolation or paired. Isolated injected animals behaved like the control solution-injected rats. Irrespective of the previous marihuana injection they fell asleep and showed a typical REM rebound. Similar results were observed in the nondeprived and paired marihuana injected animals. Conversely, when the REM deprived-marihuana injected animals were paired, they had no sleep and fought. In this connection their behavior did not differ from that of the similarly treated but not recorded animals.

These results suggest that the aggressiveness inducing properties of marihuana are related to the REM deprivation and to the increased environmental stimulation achieved by pairing the rats. REM deprivation could be contributing by increasing central neuroexcitability as shown by Cohen and Dement [3] when measuring electroconvulsive thresholds. Further, the mechanisms through which marihuana triggers aggressive behavior appear to be efficiently neutralized in nondeprived rats by other physiological mechanisms. Conversely, the absence of aggressiveness in the REM deprived-control injected and paired rats indicates that the mechanisms through which marihuana produced aggressiveness do not fire spontaneously.

Recently, it has been forwarded that during REM sleep a restoration of brain catecholaminergic systems used up during wakefulness takes place [11]. This possibility would be hampered in those animals prevented to regain REM. Further, concerning aggressive behavior it has been postulated that norepinephrine integrates a central inhibitory system as opposed to an excitatory dopaminergic one [4], and evidence from our laboratory [2] shows that pharmacological manipulations of brain catecholamines profoundly affects aggressiveness induced by marihuana in REM deprived rats. Therefore, the possibility that REM deprivation may also play a role in this form of aggressive behavior through an alteration of brain catecholamines can not be ruled out at present, and should be further investigated.

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