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Effects of REMS Deprivation on Striatal Dopamine and Acetylcholine in Rats[1]

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GHOSH, P. K., P. D. HRDINA AND G. M. LING. *Effects of REMS deprivation on striatal dopamine and acetylcholine in rats.* PHARMAC. BIOCHEM. BEHAV. 4(4) 401–405, 1976. – Changes in the concentration of striatal dopamine (DA) and acetylcholine (ACh) in rats deprived of REM sleep for 10 days were compared with those obtained after a 4 day deprivation procedure. Animals placed on small (7 cm dia.) islands surrounded by water were completely deprived of REM sleep but able to obtain some slow-wave sleep. Concentration of striatal DA was significantly increased after 4 days and 10 days of REM sleep deprivation by 73 and 133%, respectively when compared to controls. Levels of ACh in the striatum were significantly enhanced (by 28%) after 10 day, but failed to show significant change after 4 day REM sleep deprivation procedure. The short term locomotor activity was significantly higher in REM sleep-deprived animals. Our data indicate that REM sleep deprivation results in marked alterations of both cholinergic and dopaminergic mechanisms in the rat striatum.

REMS deprivation Striatal DA and ACh

ALTHOUGH it is believed that brain biogenic amines play an important role in sleep mechanisms, their exact relationship to either slow-wave or REM sleep is not clear. Jouvet [8,10] has suggested that whereas serotonergic mechanisms may be responsible for producing slow-wave sleep, norepinephrine is involved in the occurance of REM sleep periods. On the other hand, a reciprocal relationship between brain norepinephrine and REM sleep time was proposed by Hartmann [4] suggesting that REM sleep "functions to restore synaptically active brain norepinephrine and maintain the functioning of norepinephrine-dependent brain systems" rather than norepinephrine producing REM sleep. Furthermore, accumulation of brain acetylcholine during sleep has been reported by Richter and Crossland [17].

Sleep deprivation has often been used in studies examining the possible involvement of brain biogenic amines in various stages of sleep. In experimental animals, the behavioural effect of REM sleep deprivation is characterized by increased responsiveness to external stimuli, irritability and aggressiveness [9]. In this condition, an enhanced turnover of brain norepinephrine and significant decrease in brain ACh levels without concomitant alterations in levels of norepinephrine and serotonin have been reported [3, 12, 18]. In addition, Hernández-Peón *et al.* [5] found that REM sleep deprivation resulted in marked increases of dopamine in the midbrain tegmentum of cats. The aim of the present investigation was to examine whether REM sleep deprivation would alter the levels of acetylcholine and dopamine in the striatum of rats and disturb the balance between cholinergic and dopaminergic mechanisms in this brain region.

METHOD

Male Sprague-Dawley rats, weighting 160–200 g were used in this study. Deprivation of REM sleep was achieved by using the procedure described by Ling and Usher [11]. Experimental animals were isolated on small plastic islands, 7 cm in dia., completely surrounded by water. The rats were permitted non-REM sleep, but were unable to obtain REM sleep since the ensuing relaxation of the muscles of the neck would cause their heads to drop into the water. Other animals were placed on larger (12 cm in dia.) islands also completely surrounded by water. Under this circumstance, the rats were allowed to rest and obtain both non-REM and REM sleep. The experimental animals had free access to food and water. Control rats were placed in individual cages kept in the same room. The weight of animals was recorded daily. The locomotor activities of REM sleep-deprived as well as of control rats were measured daily by using a selective activity meter (Columbus Instruments, Ohio).

Two separate experiments were carried out. In one, the rats were isolated either on small (n = 12) or large (n = 10) islands for 4 days; in the other, a group of 12 rats was isolated on small islands for 10 days. The controls for the 4 day deprivation experiments consisted of 12 rats housed in separate cages and of 6 rats kept in groups, and for the 10 day deprivation procedure of 12 rats kept in separate cages.

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Upon the termination of the experiments the rats were immediately sacrificed by using near freezing technique of Takahasi and Aprison [22]. After decapitation, the brains were rapidly removed and the striata dissected. Brain dopamine was measured by the method of Spano and Neff [19] and acetylcholine was estimated by using pyrolysis-gas chromatography as described in an earlier communication [6]. The results were statistically evaluated and the significance of differences was calculated by using Student's *t* test.

RESULTS

Effects of 4 Day REM Sleep Deprivation

It was first confirmed from EEG, EOG and EMG recordings that by isolation on small islands the experimental animals were indeed deprived of REM sleep. For this purpose, 2 rats were implanted with electrodes into the dorsal hippocampus, the cortex, the external margin of the eye and in the muscles of the back of the neck in order to permit electroencephalographic (EEG), electrooculographic (EOG) and electromyographic (EMG) recordings during the above experimental procedure. An analysis of sleep pattern during a typical REM sleep deprivation experiment in the rat is reported in Table 1. Before the start of the deprivation experiment (on the last day of baseline recording), the implanted rats had an average of 15 (13 and 17) periods of REM sleep of average duration of 1.8 (1.6 and 2.0) min which accounted for 13.7 (13.5 and 13.9)% of total sleep time during 5 hr of recording. The deprivation procedure did not permit any periods of REM sleep although the animals were able to obtain numerous brief periods of slow-wave sleep. Immediately after the termination of the chronic REM sleep deprivation procedure the recording has revealed a marked rebound increase in REM sleep to 44.7% of the total sleep time (Table 1).

The differences in body weight between the control and experimental groups, expressed as percentages of the initial body weight, are shown in Fig. 1. Whereas during the first 3 days there was an almost linear weight increment in control rats housed either singly or in groups, animals isolated on large islands failed to gain any weight and the REM sleep-deprived rats (on small islands) even lost some (about 5%) of their initial body weight during the first 2 days and failed to recover it during the remainder of the experiment.

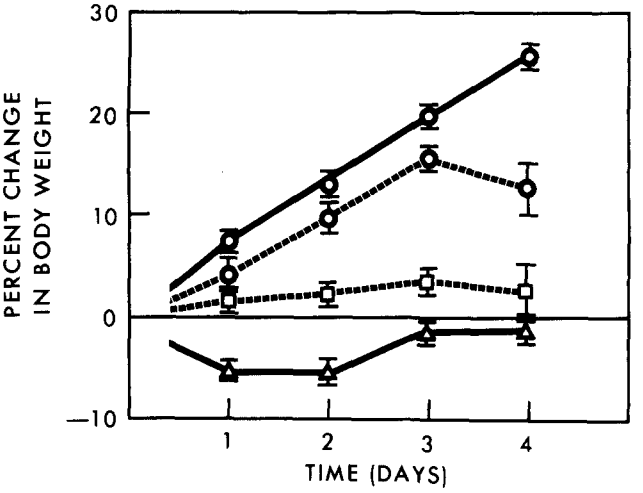

FIG. 1. Effect of 4 days of REM sleep deprivation on the percentage change in body weight of rats, taking the weight at 0 day as 100 percent. Each point represents mean value from 6 animals; vertical bars indicate SEM △ —△, REM sleep-deprived rats, □ ———□ rats placed on large (12 cm) islands; ○ ———○, control animals kept individually in separate cages; ○——○, control rats caged in groups.

Upon the termination of the 4 day REM sleep deprivation procedure the rats showed increased irritability in response to external stimuli such as hand claps, high pitch sound and attempts to handle.

Concentration of striatal dopamine was found to be markedly increased not only in the group of REM sleep-deprived animals but also in rats isolated on larger (12 cm) islands. As shown in Fig. 2, the increases represented 73 and 112%, respectively when compared to the values noted in control animals (3.2 ± 0.2 μg/g).

In contrast, levels of acetylcholine in the striatum of both experimental groups were slightly although not significantly reduced to 91 and 92%, respectively when compared with controls (44.4 ± 1.9 nmol/g).

Effects of 10 Day REM Sleep Deprivation

Changes in the body weight of rats placed for 10 days on small islands (REM sleep-deprived) in comparison with those of control animals are presented in Fig. 3. Control

TABLE 1

ANALYSIS OF SLEEP PATTERN DURING A TYPICAL CHRONIC REM SLEEP DEPRIVATION EXPERIMENT IN THE RAT

Parameters measured	Base Line Day 3	Exp. Day 1	2	3	4	7	8	9	10	11	After exp. Day 1
TWT/TRT* (%)	35.6	100	62.5	80.8	98.4	96.1	93.3	98.6	100	100	52.6
TST/TRT (%)	64.4	0	34.8	19.2	1.6	3.9	6.7	1.4	0	0	47.4
SWS/TRT (%)	55.7	0	34.8	19.2	1.6	3.9	6.7	1.4	0	0	26.2
REM/TRT (%)	8.7	0	0	0	0	0	0	0	0	0	21.2
SWS/TST (%)	86.5	0	100	100	100	100	100	100	0	0	55.3
REM/TST (%)	13.5	0	0	0	0	0	0	0	0	0	44.7
No. of REM periods	17	0	0	0	0	0	0	0	0	0	14
Average Duration of REM period (min)	1.56	0	0	0	0	0	0	0	0	0	2.71

*TWT, total waking time; TRT, total recording time; TST, total sleep time; SWS, slow wave sleep; REM, REM sleep.

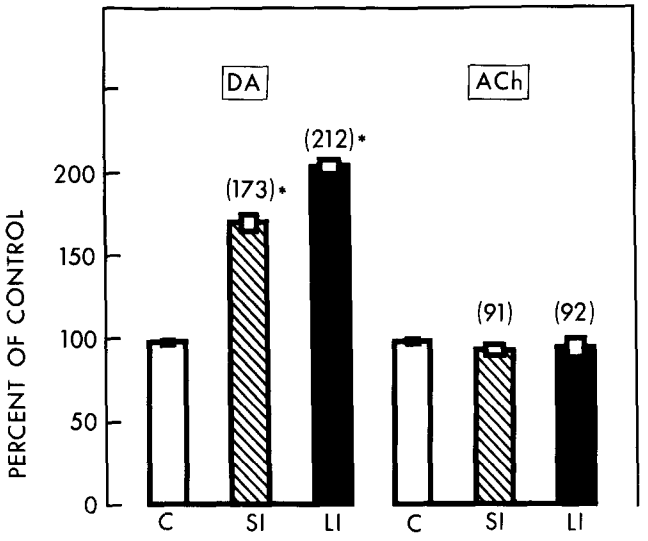

FIG. 2. Effect of 4 day REM sleep deprivation on the concentration of dopamine and acetylcholine in the rat striatum. Columns represent mean values ± SEM from 5 animals in each group. Data are expressed in percentages taking the values of control animals as 100% C, controls; SI, rats placed on small (7 cm in dia.) islands (REM sleep-deprived); LI, animals placed on large (12 cm in dia.) islands. *Statistically significant difference ($p<0.001$) when compared to the respective control group.

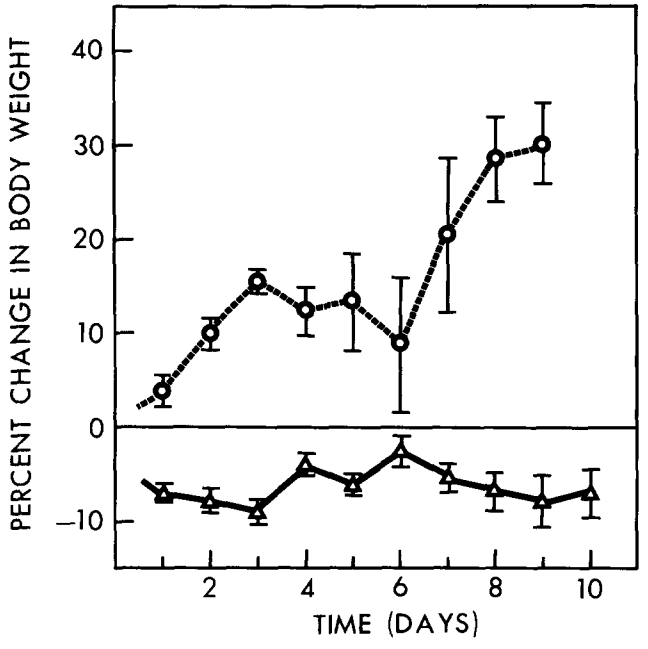

FIG. 3. Effect of 10 day REM sleep deprivation on the body weight of rats. Each point represents the mean value ± SEM of 6 animals. Data are expressed as percentages taking the weight at day 0 as 100%. △——△, REM sleep-deprived rats; ○ — — —○, controls.

rats kept in separate cages showed normal increases in body weight whereas the REM sleep-deprived animals displayed a 2 phase decrease in their weight which at the 10th day was actually below that at the beginning of the experiment.

The locomotor activity of control as well as of experimental (REM sleep-deprived) animals was measured daily at the same hour to avoid possible changes due to the circadian rhythm. As shown in Fig. 4, the locomotor activity of the control group has, after an initial fall during the first two days, stabilized around the values of approximately 200 counts per 5 min. In contrast, the locomotor activity of rats isolated on small islands (REM sleep-deprived) did not show an initial drop and was from the second day on consistently higher (by about 50%) than that of control animals.

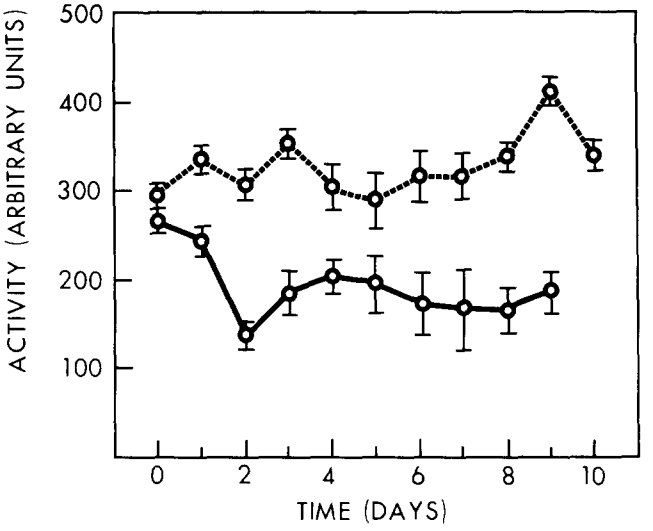

FIG. 4. Effect of 10 day REM sleep deprivation on the locomotor activity of rats. Each point represents mean value of 6 animals, vertical bars indicate SEM. ○ — — —○, rats placed on small islands (REM sleep-deprived); ○——○, control rats. Locomotor activity is expressed as number of counts per 5 min. The difference between the control and experimental group was statistically significant $p<0.05$ at all time intervals except days 0 and 5.

Levels of dopamine and acetylcholine in the striatum of rats deprived of REM sleep for 10 days in comparison with those of control animals are shown in Fig. 5. Similar to 4 day REM sleep deprivation, the concentration of striatal dopamine in rats deprived of REM sleep for 10 days was markedly increased, attaining a mean value which was 133% higher than that found in the control group (3.2 ± 0.2 μg/g). In contrast to the finding after the 4 day deprivation procedure, acetylcholine levels in the striatum of rats isolated on small islands for 10 days (Fig. 5) were significantly ($p<0.001$) higher (56.0 ± 2.5 nmol/g) than those in the control group (44.4 ± 1.9 nmol/g).

DISCUSSION

Our data indicate that REM sleep deprivation in rats results in marked alterations of both cholinergic and dopaminergic mechanisms in the striatum. Concentration of striatal dopamine was significantly increased after both 4 day and 10 day REM sleep deprivation procedure by 73 and 133%, respectively when compared to controls. Levels of acetylcholine in the striatum were significantly enhanced (by 28%) after 10 days but failed to show a significant change after 4 days of REM sleep deprivation. Results of the objective measurement of changes in locomotor activity as a function of REM sleep deprivation confirmed the earlier findings of Albert *et al.* [1] in that the REM

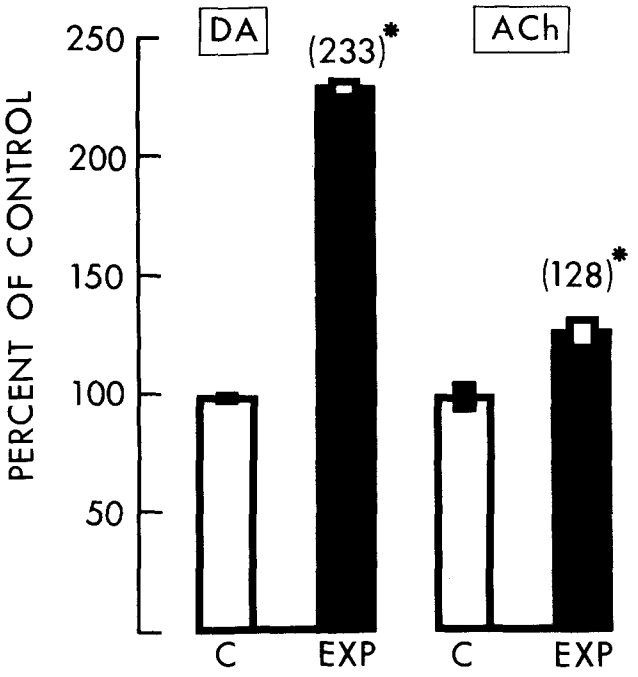

FIG. 5. Effect of 10 day REM sleep deprivation on the concentrations of dopamine and acetylcholine in the rat sriatum. Columns represent mean values ± SEM of 6 animals in each group. Data are expressed in percentages taking the values of control animals as 100%. C, controls; EXP, REM sleep-deprived rats. *Statistically significant difference when compared to control group. ($p<0.05$).

sleep-deprived animals display a substantially higher short term activity than the control rats due probably to the sensitization to environmental stimuli [14].

One of the approaches to elucidating the possible role of biogenic amines in various stages of sleep has been the investigation of neurochemical consequences of total and REM sleep deprivation. REM sleep deprivation in rats was found to result in increased synthesis [21] and utilization [18] as well as enhanced turnover [12,16] of brain norepinephrine. Schildkraut and Hartmann [18] reported that endogenous levels of norepinephrine in the whole brain of rats remain unchanged after 72 hr of REM sleep deprivation and Bliss [2] found no significant changes in the brain content of this amine even when the deprivation procedure was extended to 10 days. However, Tsuchiya *et al.* [23] found marked decreases in the concentration of norepinephrine in the diencephalon and mesencephalon of rats deprived of REM sleep for 96 hr. On the other hand, Stern *et al.* [21] in a study of regional levels of brain amines after 3, 6 or 8 days of REM sleep deprivation in rats failed to detect any significant alterations of norepinephrine content in the cerebral hemispheres, diencephalon or brain stem.

Bowers *et al.* [3] reported that rats deprived of REM sleep (and partially of slow-wave sleep) show a significant fall (by 35%) in the amount of acetylcholine in telencephalon without any concomitant changes in diencephalon and brain stem. Reduction of acetylcholine levels in the striatum of rats deprived of REM sleep for 96 hr found in our experiments was similar to that reported by Tsuchiya *et al.* [23] for telencephalon, a brain area including cortex, caudate and hippocampus. It is of interest that the latter authors found an opposite change, i.e. an increase of acetylcholine levels in the telencephalon of rats after 24 hr of total sleep deprivation.

Alterations in striatal acetylcholine found in our experiments after 10 days of REM sleep deprivation were different from those observed after the 4 day deprivation procedure. It has been reported [7] that during REM sleep the cortical release of acetylcholine (which is suppressed during the slow-wave sleep) rises to waking levels. The possibility thus exists that chronic REM sleep deprivation may result in reduction of acetylcholine release and lead to accumulation of this neurohormone in brain tissue. Another possible explanation may be derived from observation of Tsuchiya *et al.* [23] that in rats deprived of total sleep the telencephalic levels of acetylcholine were increased. The deprivation of REM sleep in last days of our chronic deprivation experiment might have been accompanied by total sleep deprivation as well, as it was in the typical experiment analysed in Table 1.

Although it has been reported that the administration of the precursor of dopamine, L-DOPA suppresses REM sleep in man [15,20], little attention has been paid to the possible role of brain dopamine in sleep mechanisms and only a few reports deal with measurement of this amine during REM sleep deprivation [2,5]. Whereas Bliss [2] found no changes of dopamine content in the whole brain of rats deprived of REM sleep for 10 days, Hernández-Peón *et al.* [5] reported a marked increase in the levels of this amine in the midbrain tegmentum of cats after 12 days of REM sleep deprivation. Our findings of increased dopamine concentration in the striatum of rats deprived of REM sleep for either 4 or 10 days is in agreement with the latter observation. However, similar increases of striatal dopamine were noted in rats placed on large (12 cm) islands; in this condition the animals may also be partially deprived of their REM [18] and/or slow-wave [13] sleep. This, of course raises the question of whether the biochemical changes observed after sleep deprivation procedures can be considered as a specific consequence of REM and/or total sleep deprivation or result rather from concomitant stress factors. Opinions on this important question are divided. Some workers [12,18] suggested that the increase in norepinephrine turnover as well as in the weight of adrenal glands may be entirely a result of the experimental stressful situation, since these changes were similar in rats kept on small islands (completely REM sleep-deprived) and on large platforms. Our findings of increased striatal dopamine content in both experimental groups would concur with the above suggestion. On the other hand, Bowers *et al.* [3] and Tsuchiya *et al.* [23] felt that the alterations in brain acetylcholine observed in REM sleep-deprived rats are an indication of metabolic changes due to REM deprivation and cannot be considered a nonspecific response associated with stress factors, since other stressful situations (food deprivation, restraint) did not lead to changes in telencephalic acetylcholine values. Methods have yet to be devised to elucidate whether changes in the levels and metabolism of various brain biogenic amines are a specific consequence of REM and total sleep deprivation or reflect a response to a general stressful situation.

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