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Disruption of Passive Avoidance Memory by REM Sleep Deprivation: Methodological and Pharmacological Considerations

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HARRIS, P. F., D. H. OVERSTREET AND J. ORBACH. *Disruption of passive avoidance memory by REM sleep deprivation: Methodological and pharmacological considerations.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1119–1122, 1982.—The present experiments were designed to examine more closely the variables responsible for the disruption of passive avoidance memory produced by REM sleep deprivation. In the pharmacological study it was found that imipramine could reverse the memory disruption exhibited by rats maintained on large platforms (presumably not REM-deprived) while both imipramine and physostigmine were required to reverse the memory disruption exhibited by rats maintained on small platforms. In the methodological study it was found that those animals maintained on the smallest platforms and therefore having the largest weight to area ratio exhibited the greatest degree of memory disruption. It is concluded that further modification and verification of the platform techniques of REM deprivation is required before firm conclusions about its neurochemical basis and behavioural functions can be made.

REM sleep deprivation memory disruption Physostigmine Imipramine Weight to area ratio

SINCE the discovery of the rapid eye movement (REM) state of sleep [1], numerous controversies have arisen. These have included arguments about the precise function(s) of REM sleep [3, 10, 16, 21], the neurochemical mechanisms underlying it [6, 7, 8, 9, 21] and the methodological difficulties associated with attempts to deprive rodents of REM sleep [2, 7, 11, 13, 14, 15]. The most common manner used in these latter studies has been the "flower pot," or the more preferred term of the "platform" technique [5,12], whereby a rat is placed on an inverted flower pot surrounded by water; because of the muscle hypotonia at the onset of REM sleep the rat's head or body contacts the water and it either wakes or returns to slow wave sleep. Among the problems with this technique are dissociating the effects of stress, sleep stage specificity and other variables from those of REM sleep deprivation itself and obtaining some reliable index by which to compare the many studies in this field [12,22]. In the present communication we wish to report some recent findings from our laboratory which indicate that continued modification, verification and standardization of the techniques for REM sleep deprivation are required before any conclusions about its neurochemical correlates can safely be made.

EXPERIMENT 1

Several years ago it was reported that physostigmine, a reversible anticholinesterase agent, reversed the memory-disruptive effects of REM sleep deprivation on a passive avoidance task [20]. This finding was at variance with the predictions of a prominent theory of the neurochemical basis of REM sleep [21], which implied that only catecholamine agonists could reverse this behavioural deficit. More recent studies in humans support the concept of a close relationship between REM sleep and the cholinergic system [19]. Numerous studies in this area, including that by Skinner *et al.* [20], have suffered from methodological weaknesses; one of the most important was the failure to use an adequate control group, specificially a large platform group. In a preliminary experiment designed to overcome this deficiency, it was found that rats placed on large (11.5 cm) platforms also exhibited disruption of memory for a passive avoidance task (Harris, unpublished honours thesis). The present experiment was designed to explore more fully the pharmacological aspects of the reversal of memory disruption produced by the platform conditions. Imipramine, a catecholamine reuptake blocker, was able to reverse the memory disruptive effects of the large platform condition, while both imipramine and physostigmine were required to reverse the memory disruptive effects of the small platform condition.

Method

Naive male Hooded Wistar rats, 90–120 days old and weighing between 200 and 350 g, were used in this experiment. They were housed in black plastic cages in groups of four with free access to food and water.

The REM sleep deprivation apparatus consisted of three 44×31×23 cm waterproof fibreglass tanks. Wire mesh ceilings were positioned 11.5 cm above the rats to prevent them from escaping. Through these ceilings water bottles and food were continuously available. The water in the tanks was 11

cm deep and came within 0.5 cm of the top of the platforms and was heated to 25°C.

The diameters of the platforms were 7.0 cm for the REM-deprived group and 11.5 cm for the control group. These were selected on the basis of previous verification studies [13,14].

All rats were maintained on the large platform for 24 hr under continuous lighting. After this adaptation period, the rats were randomly assigned to either a large or small platform for three days.

A modified one-trial passive avoidance task was used. The animals were trained in a Y maze to remain in a lighted, unshocked alley immediately after the completion of the REM deprivation phase. Three measurements of acquisition were taken: the time taken for the rats to leave the lighted alley; the number of mistakes made (shocks received) before the task was learned; and the total time taken to reach the criterion of learning, which was to remain in the lighted alley for at least one min. To test for retention, the rats were placed in the lighted alley 72 hr and later the time taken to leave was recorded (up to a maximum of 5 min). During the training-retention period the rats were returned to group cages.

The cholinergic drugs were physostigmine sulphate (0.05 mg/kg), an anticholinesterase agent, and scopolamine methyl nitrate (4 mg/kg), an anticholinergic with predominantly peripheral actions. Imipramine, (5 mg/kg) which inhibits the uptake of noradrenaline and serotonin into their presynaptic terminals, was chosen as a catecholamine potentiating agent [21]. All drugs were dissolved in isotonic saline and administered IP in volumes of 1 ml/kg. The dosages of the drugs refer to their respective salts. Methyl scopolamine or imipramine were administered immediately after the completion of the training, while physostigmine or saline were administered 20 min later.

Nonparametric statistics [18] were used to analyse the data after it was determined that the variances among the groups were not homogeneous.

Results and Discussion

There were no significant differences among the groups for indices of acquisition; i.e. the time taken to leave the safe alley, the number of mistakes, or the time to criterion which was to remain in the lighted alley for one min. (cf [20]). The results for the retention test are summarised in Table 1. It can be seen that memory disruption was evident in groups maintained on either the large or small platform. However, while imipramine treatment itself was sufficient to reverse the effects of the large platform condition, both imipramine and physostigmine administration were required to reverse the effects of the small platform condition.

These findings are consistent with the hypothesis that stress may be involved in the disruptive effect of the platform technique, because both platform conditions have been shown to produce comparable increases in adrenal weights (Harris, unpublished observations). Furthermore, this result is compatible with the work of Mark *et al.* [11], who found that the increased turnover of noradrenaline for both the experimental and control group was not a direct consequence of REMD but a stress reaction. Also, the fact that rats both platform conditions exhibited an adrenal hypertrophy when compared with rats from group cages supports the assumptions of Morden *et al.* [14] in their original verification study regarding the function of the large platform.

TABLE 1

MODIFICATION OF THE MEMORY-DISRUPTIVE EFFECTS OF THE PLATFORM TECHNIQUE BY IMIPRAMINE OR PHYSOSTIGMINE

Platform Condition*	Pharmacological Treatment	Retention Score†
Group Cage Control	MS + SAL	300 ± 142
Group Cage Control	MS + I	300 ± 0
Large platform	MS + I	300 ± 55
Large platform	MS + P	19 ± 16‡
Large platform	MS + I + P	300 ± 0
Small platform	MS + I	23 ± 2‡
Small platform	MS + P	27 ± 124‡
Small platform	MS + I + P	300 ± 0

*Rats were housed in groups of four or individually on the large (11.5 cm) or small (7.0 cm) platforms for four days before being trained in a Y maze for a passive avoidance task.

†Median ± semi-interquartile range.

‡Significantly different from Group Cage Controls, $p<0.05$, Mann-Whitney U tests for independent samples.

Nevertheless, the fact that rats in both conditions exhibit stress reactions suggests an inherent methodological fault in the use of the technique. It should be noted that Stern and Morgane did not employ a large platform control but rather a water immersion control which has been reported to cause both behavioural performance deficits and catecholamine depletion [23,24].

The fact that imipramine, a catecholamine reuptake blocker, reversed the disruptive effects of the large platform condition, suggests that catecholaminergic systems may mediate this stress (see [23,24]). However, since both imipramine and physostigmine were required to reverse the effects of the small platform condition, both catecholaminergic and cholinergic systems must be involved. This result contradicts Stern and Morgane's [21] catecholamine maintenance hypothesis as it has only assigned a mechanistic role to the cholinergic system in REM sleep.

Although this parsimonious hypothesis implicates the catecholaminergic systems in mediating the stressfulness of the platform technique and the cholinergic system in mediating the specific effects of REM sleep deprivation, there are still several uncertainties. Because slow wave sleep may be disrupted in both platform conditions, the serotonergic system may also be involved in mediating these effects, even though this seems unlikely. It is also possible that the animals were not given sufficient time to adapt to the conditions [4], and that the memory-disruptive effects may be related to this factor. Preliminary findings indicate that only a single day on the platforms can lead to disruptive effects of acquisition of the passive avoidance task as well as memory disruption. Further studies on the adaptation to the platforms are now in progress.

Because this research was completed prior to the publication of the review of Hicks *et al.* [5], the methodological oversight emphasized in their review also applies to the present experiment. Because the weights of the rats varied widely, the groups may not have been differentially treated with regard to REM sleep deprivation. It is also apparent that they were not differentially treated with regard to stress which is reflected by adrenal hypertrophy. To explore this

methodological question further, the second experiment was conducted.

EXPERIMENT 2

Two recent reviews have proposed that the weight to area (W/A) ratio (the weight of the rat divided by the area of the platform) may be a suitable variable for comparing different studies which have employed the platform technique [5,12], to ascertain whether the groups were differentially treated. Unfortunately, many of the earlier studies failed to report both weights of their animals and sizes of their platforms or used conditions which did not adequately control for the platform technique [12]. In a *post hoc* analysis, McGrath and Cohen [12] interpreted Mendelson *et al.*'s [13] data to calculate the appropriate W/A ratios for the experimental and control platforms (6.5 and 1.7 respectively). Hence the use of these W/A ratios with approximately the same weighted animals should ensure differential treatment of the groups after 96 hours. In the present experiment strict adherence to (W/A) ratios was maintained in order to determine the relationship between this variable and the performance on a passive avoidance task.

Method

Male Sprague-Dawley rats, approximately 90 days old and weighing 200–350 g, were used. They were housed in groups of six under a 12:12 light regime with free access to food and water.

The REM sleep deprivation apparatus consisted of a single wooden box measuring 145×84×60 cm deep. A plastic tank was placed inside the outer tank and this was divided into six REMD chambers measuring 31×31×46 cm deep.

The floor of the chambers consisted of a sheet of aluminum on which the platform stems were mounted. The stems were located in the centre of each compartment with the platform 15 cm above the floor level. Water came to within 0.5 cm of the platform top. A wire mesh ceiling was placed 15 cm above the platform top. Through this, food was available ad lib.

A water heater was placed in one end of the tank to ensure that the water temperature was maintained at 25°C. This was done to control for the exposure to chronic enforced cold water swims which are known to cause behavioural deficits [23].

On the underside of the tank lid six lights were positioned so as to illuminate each chamber. An exhaust fan was mounted in the lid with adjacent holes to ensure a continual flow of air to the tank.

All animals used were given 2 days habituation to the platform condition at least 4 days prior to the commencement of the experiment. For this adaptation phase, the animals were wieghed and W/A 1.7 platform size calculated. The animals were placed on the platforms for 48 hours and then returned to the home cage until the commencement of the experiment, which was at least 7 days later.

There were five groups in this experiment, four W/A ratio platform conditions and a group cage control. The four platform conditions included W/A 4.0 and 8.0 as well as the control and experimental ratios proposed by McGrath and Cohen [12].

During the REM deprivation phase the animals were maintained on their respective platforms for 96 hours. They were removed for ½ hr after 48 hr. Training and retention procedures were the same as in Experiment 1.

TABLE 2

THE RELATION BETWEEN WEIGHT TO AREA RATIO AND MEMORY DISRUPTION OF A PASSIVE AVOIDANCE TASK

Pretraining Platform	N	Retention Score Mean ± Standard Error
Group cage control	7	191.5 ± 51.5
W/A 1.7	6	102.8 ± 35.8
W/A 4.0	6	44.6 ± 31.3
W/A 6.5	9	20.4 ± 5.3*†
W/A 8.0	6	15.6 ± 4.2*†

*Significantly different, $p<0.05$, from the group cage control; Student's t-test.

†Significantly different, $p<0.05$) from W/A 1.7 condition; Student's t-test.

Results were initially analyzed by one way analysis of variance. When this was significant, follow-up Student's t-tests were carried out.

Results and Discussion

Again, no significant differences among the groups were found for the parameters of acquisition. The results of the retention test are summarised in Table 2. The best memory was exhibited by the group cage control, while the poorest memory was exhibited by the two groups which had the highest W/A ratios. These two latter groups also had significantly poorer memories than the group with the smallest W/A ratio.

These experiments incorporated a pretraining REM sleep deprivation paradigm in that the rats were REM deprived prior to training. According to Dewan's [3] programming hypothesis, REM sleep deprivation prior to training should cause deficits in the acquisition of the task. No acquisition deficits were observed for any platform groups.

However, Pearlman and Greenberg [16] have modified Dewan's hypothesis on the basis of Seligman's [17] differentiation between prepared and unprepared tasks. As a consequence, they have suggested that pretraining REMD would only disrupt the acquisition of tasks not related to survival. Pearlman and Greenberg [17] have hypothesized that prior REM sleep deprivation would only affect the synthesis of novel information. According to this criterion, the modified passive avoidance task employed in this study would not have been expected to produce any acquisition or retention deficits. However, retention of the task 3 days later was affected in the two platform conditions presumably exhibiting significant REM sleep deprivation; i.e., W/A 6.5 and 8.0. This result is at variance with both Dewan's and Pearlman and Greenberg's hypotheses concerning a pretraining paradigm.

These findings reinforce the arguments made by McGrath and Cohen [12] that a W/A ratio of 1.7 may be suitable for a control platform condition, while a W/A ratio of 6.5 would be suitable for the REM sleep deprivation condition. However, it has not been established how W/A ratios are related to the weight of the rat. As some groups in the present experiment contained rats weighing in excess of 300 g and the W/A ratios of McGrath and Cohen [12] were calculated for animals

weighing 200–225 g, it is important to establish that a given W/A ratio will produce an amount of REM sleep deprivation that is independent of the weight of the rat.

It is also important to note that the retention score of the W/A 1.7 group, although not significantly different from the group cage control (Table 2), was not perfect. However, it was substantially better than a large platform group used in the first experiment (Table 1), when an habituation phase was not used. If an habituation phase of four days had been employed [4], the W/A 1.7 group may have exhibited an even better memory.

GENERAL DISCUSSION

The results presented in these experiments are not consistent with contemporary hypotheses concerning the functional significance of REM sleep [3,16] nor its neurochemical basis [21]. Because these results are at variance with predictions from contemporary theories, studies are in progress to:

(1). Determine the amount of stress associated with each condition by measuring adrenal weight, blood corticosterone and stomach ulceration.

(2). Assess the role of adaptation to the platform technique in producing the disruptive effects on memory.

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