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BRIEF COMMUNICATION

The Flower Pot Technique of Rapid Eye Movement (REM) Sleep Deprivation

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MENDELSON, W. B., R. D. GUTHRIE, G. FREDERICK AND R. J. WYATT. *The flower pot technique of rapid eye movement (REM) sleep deprivation.* PHARMAC. BIOCHEM. BEHAV. 2(4) 553–556, 1974. – This technique of REM sleep deprivation may make data interpretation difficult because it can lack selectivity, and because controls may suppress some REM sleep. To correct these difficulties, EEG recordings were made of rats placed in 4 situations for 96 hours: (1) baseline, (2) on 6.5 cm, or (3) 12.5 cm inverted flowerpots surrounded by water, (4) swimming in 10 cm water for 1 hr per 24 hr. Rats on the 6.5 cm pots had 57% as much REM sleep as baseline with no change in non-REM sleep. Rats on 12.5 cm pots initially had 55% as much REM sleep as baseline, but by the fourth day increased to baseline levels. The swimming rats had no reduction in REM or non-REM sleep at any time, and thus seem to be a better control. The smaller the platform relative to the size of the rat, the greater the reduction in REM sleep – but at one point, non-REM sleep is decreased. The combination used here depresses REM sleep by about one half but does not reduce non-REM sleep.

REM sleep deprivation Flower pot technique Sleep

PERHAPS the most obvious way to produce Rapid Eye Movement (REM) sleep deprivation is to follow the electroencephalogram (EEG) and arouse the subject at the onset of stage REM. This approach is inefficient – it requires constant monitoring and may necessitate arousing an animal up to 134 times over a 24 hr period [9]. In 1964, Jouvet *et al.* [5] suggested a technique more suitable for animal experimentation. Cats were placed on small pedestals surrounded by water. The general somatic relaxation associated with REM sleep caused the cat to fall in the water and thus aroused it. In 1965 Cohen and Dement [1] adapted this approach for rats. Duncan *et al.* [2] and Pujol *et al.* [10] provided EEG data on rats treated in this manner.

Since this time there have been a variety of studies on the behavioral and biochemical effects of REM sleep deprivation, based on the flower pot technique [1, 3, 4, 6]. We are concerned because it seems to have two pitfalls:

(1) Under certain conditions, it may not be selective – in addition to depriving rats of REM sleep, non-REM sleep may be significantly reduced. One paper on REM deprivation and serotonin metabolism [4], for instance, is based on a study [10] in which REM sleep is virtually abolished, but non-REM sleep is also reduced to 58.4% of control values. Because two variables are changed, the results are less clear than they might have been if only REM sleep were reduced.

(2) There has been difficulty in finding an adequate control situation – one which simulates the experimental condition, but does not reduce REM sleep. Some studies [2,4] use rats on a larger pedestal as a control. In principle, these rats will have room to sleep with general muscular relaxation and thus have normal amounts of stage REM. Duncan *et al.* [2], however, pointed out that rats on a large

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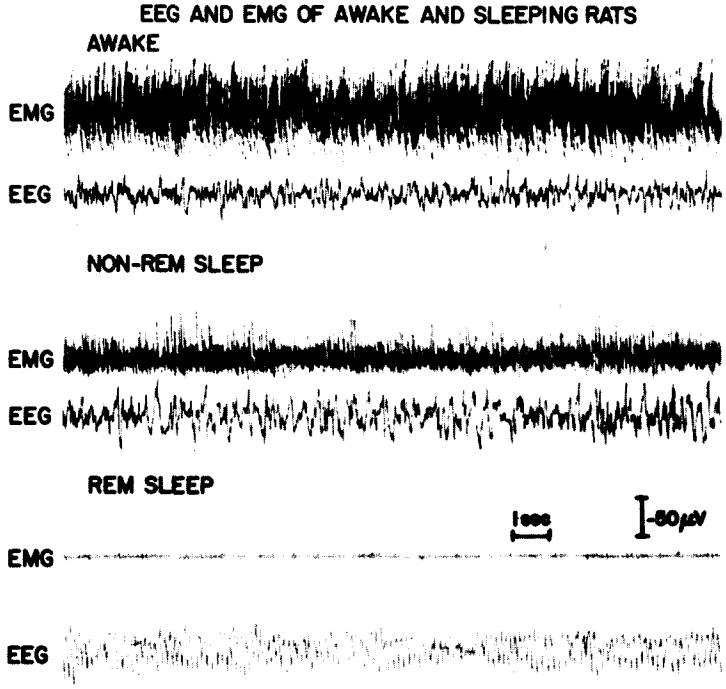

FIG. 1. Polygraphic recordings of rats when awake, in non-REM and REM sleep.

12 cm platform were indeed deprived of 50 percent of their REM sleep over five days. Thus to use the larger pot for a stress control would seem to make data interpretation difficult, as REM sleep is also deprived. An interesting alternative has been used by Stern *et al.* [12]; rats were placed in 10 cm of water to swim for one hr per 24 hr.

In an effort to develop an adequate and selective method of REM sleep deprivation for our neurochemical studies, we have done EEG's on rats in normal cages and two sizes of flower pots. In order to document a control which does not deprive rats of REM sleep, we have recorded EEG's of rats who swam for one hour per day. Gross measures of comparability of stress have been provided for all four groups.

METHOD

Male Sprague-Dawley rats, weighing 200–225 g, were used. After anesthesia with subcutaneous pentobarbital 40 mg/kg, 3 electrodes made from stainless steel screws (0–80, 1/8 in.) were installed on the fronto-parietal cortex. Two nuchal muscle electrodes made of 28 ga copper magnet wire were installed. Animals were allowed to recover and acclimatize for one week in a soundproof experimental room. A light-dark cycle was maintained with lights on from 6 a.m.–6 p.m. Until 48 hr before the experiment, animals ate NIH rat and mouse feed [7] ad lib. Starting 48 hr before the experiment and for its duration they were allowed to feed ad lib up to 100 cc per 24 hr of a mixture of Metrecal-Shape (Mead Johnson Distributors) adjusted to give 100 Kcal/gm. The effects of this diet on weight and CNS metabolism are discussed by Veech *et al.* (in preparation).

There were four experimental conditions, to which independent groups of rats were subjected: (1) Baseline: rats lived singly in sawdust-lined containers 25 cm in diameter. (2) REM sleep-deprived: rats placed on inverted flower pots 6.5 cm in diameter, surrounded by water in a 25 cm diameter container. The water level was within 1 cm of the top of the flower pot. In this group and the 2 controls, water temperature was maintained at 19°C. (3) Traditional control: rats placed on 12.5 cm inverted flower pots surrounded by water to within 1 cm of top of pot. (4) Swimming group: rats placed in 10 cm of water from 4 p.m. to 5 p.m. daily. For the remaining 23 hr they lived singly in sawdust-lined containers.

Three sets of experiments were performed. In the first, 6 rats each were placed in the 4 experimental conditions and recorded for the first 24 hr. In the second, similar numbers of rats were kept under the 4 experimental conditions for 96 hr and recordings were made for the 24 hr period between 72–96 hr. In the third experiment, rats were subjected to the experimental conditions for 96 hr, then placed in sawdust-lined cages for a rebound period; recordings were made for the first 24 hr of this period.

Recordings were made on a Grass Model 78 polygraph with a paper speed of 10 mm/sec and calibrated for 50 μv to produce a 10 mm deflection. The recordings were read in 30 sec epochs in a blind manner by three investigators. Independent readings of 4 tracings showed an interclass correlation coefficient of 0.99 for REM sleep and 0.91 for non-REM sleep [13]. Tracings were classified as either awake, non-REM sleep or REM sleep using criteria described by Morden *et al.* [9]. Figure 1 shows typical recordings and interpretations of the three states.

TABLE 1

MEASURES OF COMPARABILITY OF STRESS

	Baseline (N=8)	6.5 cm pot (N=8)	12.5 cm pot (N=8)	Swimming Rats (N=8)	Significance
Wt. of both adrenals ± SEM (mg)	27.96 ± 2.47	29.71 ± 4.58	29.66 ± 2.59	31.27 ± 3.54	NS
Wt. change after 96 hr ± SEM (g)	2.0 ± 5.5	−10.0 ± 8.1	−13.5 ± 3.7	−5.8 ± 5.9	NS
Food intake over 96 hr ± SEM (cc)	288.8 ± 19.4	227.4 ± 23.8	245.6 ± 27.0	304.0 ± 13.4	NS

The rats were weighed at the beginning and end of each experiment. Their daily food consumption was recorded. At the end of the experiment the animals were sacrificed; the adrenals were removed and weighed [8,12]. Any animal with grossly apparent pneumonia was eliminated from the study.

Statistical comparison of the four groups was done by one-way analysis of variance; comparison of any two groups was done by the Least Significant Difference Test [11].

RESULTS

Table 1 indicates some general measures of the comparability of the stress which the rats experienced. The food intake of the 3 groups over 96 hr of experimental conditions was similar. The baseline animals gained about 2 g during the experiment; the other 4 groups lost up to 13 g, but the difference was not significant on statistical analysis with this n. The weight of adrenals did not differ among the 4 groups.

Table 2 summarizes the polygraphic data. Total (REM and non-REM) sleep was indistinguishable among all 4 groups on the first and fourth days, and first day of rebound. During the first 24 hr the rats on 6.5 cm pots had significantly less (57 percent) REM sleep than the baseline group. Similarly, the rats on 12.5 cm pots had only 55 percent as much REM sleep as the baseline group. On the other hand, the swimming and baseline groups had similar amounts of REM sleep. The percentage of REM sleep to total sleep was the same in all four groups. On the fourth 24 hr period the rats on 6.5 cm pots continued to have markedly decreased REM sleep. The 12.5 cm pot group, however, appeared to have adapted to their evironment, and had as much REM sleep as the swimming and baseline groups. Again the percentage of REM sleep to total sleep remained the same among the four groups. During the first 24 hr after the rats were taken off the flower pots, the 6.5 cm pot group significantly increased REM sleep time by 280 percent, and also increased in percentage of REM sleep. There was no significant increase in REM sleep time in the 12.5 cm pot or swimming groups during this 24 hr period. In case there might be a brief rebound effect obscured by data for the full 24 hr, REM sleep time during the first eight hours of this period was evaluated – again the control groups did not differ significantly from baseline values.

DISCUSSION

The purpose of this study was to evaluate the flower pot technique as a model of REM sleep deprivation in order to help interpret previous neurochemical studies and design future ones. From the data presented, several conclusions seem justified. First of all, non-REM sleep over 24 hr did not significantly vary among the 4 groups at any time measured; this suggests that using this combination of rat and platform size, the model is fairly selective as a method of REM sleep deprivation. The similar food intake and adrenal weights of all four groups imply that the controls provide a comparable situation to the 6.5 cm pot rats. The three experimental groups had a non-significant trend to lose more weight than the baseline group, but weight loss among the 6.5 cm pot, 12.5 cm pot and swimming rats was indistinguishable.

Comparison of our data with previous work is hampered by the variability of the animals used, and the reporting of data in which rats were recorded only after they have been taken off the flower pots. Morden *et al.* [9] provide a detailed polygraphic study, but their data is not truly comparable, as the rats were kept on the pots for 16 hr per 24 hr, and then placed in normal cages for recording for 8 hr per 24 hr. Particularly relevant to the development of an adequate control is the study by Duncan *et al.* [2]. They placed rats of unspecified weight on 12 cm platforms, and found REM sleep reduced by 50%. In the present study, the rats on 12.5 cm pots were deprived of 55 percent of REM sleep during the first 24 hr. Since the swimming group, as used by Stern *et al.* [12], had no reduction in REM sleep at any time, we feel that it is a better control situation.

Relevant studies regarding the selectivity of REM sleep deprivation include that of Pujol *et al.* [10], who placed 250–280 g Charles River rats on 4.5 cm platforms. They were found to have no REM sleep, and 58.4 percent of control values for non-REM sleep. Hartmann and Stern [3] placed 200–250 g rats on a 6 cm platform, and found that REM sleep was virtually abolished, but non-REM sleep was reduced by 10–40%. In our study, rats on a 6.5 cm plat-

TABLE 2

SLEEP EEG DATA

	Total (REM + non-REM) Sleep	REM Sleep	non-REM Sleep	$\frac{\text{REM Sleep}}{\text{REM + non-REM Sleep}} \times 100$
First 24 Hr				
Baseline (N=7)	669.7 ± 54.7	106.1 ± 7.9	563.6 ± 53.4	16.6 ± 2.0
6.5 cm pot (N=5)	633.7 ± 45.1	60.2 ± 6.6	582.5 ± 43.7	9.6 ± 1.3
12.5 cm pot (N=6)	542.7 ± 37.8	58.5 ± 12.8	484.2 ± 32.4	10.5 ± 2.2
Swimming Group (N=4)	672.1 ± 39.0	99.5 ± 22.1	572.6 ± 48.6	15.0 ± 3.6
Significance	NS	$p<0.05$	NS	NS
Fourth 24 Hr				
Baseline (N=7)	669.7 ± 54.7	106.1 ± 7.9	536.6 ± 53.4	16.6 ± 2.0
6.5 cm pot (N=5)	509.5 ± 50.3	46.6 ± 19.2	462.9 ± 47.3	8.5 ± 4.0
12.5 cm pot (N=4)	578.4 ± 45.4	105.6 ± 17.6	472.8 ± 40.8	18.3 ± 2.6
Swimming Group (N=6)	607.8 ± 41.1	110.8 ± 16.2	496.8 ± 29.1	17.9 ± 1.7
Significance	NS	$p<0.05$	NS	NS
First 24 Hr of "Rebound" Period				
Baseline (N=7)	669.7 ± 54.7	106.1 ± 7.9	563.6 ± 53.4	16.6 ± 2.0
6.5 cm pot (N=6)	829.8 ± 53.6	296.5 ± 24.4	533.2 ± 42.0	35.5 ± 2.0
12.5 cm pot (N=4)	695.4 ± 105.5	132.4 ± 50.5	563.0 ± 56.6	17.0 ± 4.8
Swimming Group (N=5)	649.1 ± 63.5	96.5 ± 11.4	552.6 ± 67.5	15.6 ± 2.6
Significance	NS	$p<0.001$	NS	$p<0.001$

Values in min ± SEM

form had 57% as much REM sleep as baseline animals, with no reduction of non-REM sleep. The implication of a comparison of these studies seems to be that the smaller the platform relative to the size of the rat, the greater the reduction in REM sleep – but at one point, non-REM sleep also begins to be reduced. When this happens, biochemical data generated by the study becomes difficult to interpret, as two different sleep variables have been changed. The particular combination of rat and platform size employed in the present study reduces REM sleep by about half, but causes no reduction in non-REM sleep.

REFERENCES

1. Cohen, H. B. and W. C. Dement. Sleep: Changes in threshold to electroconvulsive shock in rats after deprivation of "paradoxical" phase. *Science* **150:** 1318–1319, 1964.
2. Duncan, R. II, P. Henry, V. Karadzíc, G. Mitchell, T. Pivik, H. Cohen and W. Dement. Manipulation of the sleep-wakefulness cycle in the rat: a longitudinal study. *Psychophysiol.* **4:** 379, 1968.
3. Hartmann, E. and W. C. Stern. Desynchronized sleep deprivation: Learning deficit and its reversal by increased catecholamines. *Physiol. Behav.* **8:** 585–587, 1972.
4. Hery, F., J. F. Pujol, M. Lopez, J. Macon and J. Glowinsky. Increased synthesis and utilization of serotonin in the central nervous system of the rat during paradoxical sleep deprivation. *Brain Res.* **21:** 391–403, 1970.
5. Jouvet, D., P. Vimont, F. Delorme and M. Jouvet. Etude de la privation sélective de la phase paradoxale de sommeil chez le chat. *C. R. Soc. Biol. Lyon* **158:** 756–759, 1964.
6. Karadzíc, V. and B. Mrsulja. Deprivation of paradoxical sleep and brain glycogen. *J. Neurochem.* **16:** 29–34, 1969.
7. Knapka, J. J. NIH Memorandum of October 5, 1971, issued by Nutritionist, Laboratory Aids Branch.
8. Mark, J., L. Heiner, P. Mandel and Y. Godin. Norepinephrine turnover in brain and stress reactions in rats during paradoxical sleep deprivation. *Life Sci.* **8:** 1085–1093, Part I, 1969.
9. Morden, B., G. Mitchell and W. Dement. Selective REM sleep deprivation and compensation phenomena in the rat. *Brain Res.* **5:** 339–359, 1967.
10. Pujol, J. F., J. Mouret, M. Jouvet and J. Glowinski. Increased turnover of cerebral norepinephrine during rebound of paradoxical sleep in the rat. *Science* **159:** 112–114, 1968.
11. Snedecor, G. W. and W. G. Cochran. *Statistical Methods.* Ames, Iowa: Iowa State University Press, 1967, pp. 272–275.
12. Stern, W. C., F. P. Miller, R. H. Cox and R. P. Maickel. Brain norepinephrine and serotonin levels following REM sleep deprivation in the rat. *Psychopharmacologia* **22:** 50–55, 1971 (BERL.)
13. Winer, B. J. *Statistical Principles in Experimental Design.* New York: McGraw Hill, 1962, pp. 124–132.