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BRIEF COMMUNICATION

A Method for Sleep Depriving Rats

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STEFURAK, S. J., M. L. STEFURAK, W. B. MENDELSON, J. C. GILLIN AND R. J. WYATT. *A method for sleep depriving rats.* PHARMAC. BIOCHEM. BEHAV. 6(1) 137–139, 1977. – A method is described for sleep depriving up to 12 rats at a time by placing them in two large rotating cylinders. EEG data, previously unavailable for rats treated in this manner, show that total sleep time was significantly reduced from 47.0 percent to 3.8 percent of a 24-hr period. There was no selective reduction of REM or non-REM sleep.

Sleep deprivation REM sleep Sleep Rats EEG Baseline Mammal

A VARIETY of methods have been employed to produce total sleep deprivation in small animals. These include continuous handling [1], the use of treadmills [6], rotating wheels [11] or small platforms [2,3] surrounded by water, administration of amphetamines [6] or electric shocks [5,9], and hyperthermia [10]. Unfortunately, these techniques are largely without support of EEG data. Most also have clear disadvantages. The use of small platforms surrounded by water is an outgrowth of a procedure for REM sleep deprivation; when used for purposes of total sleep deprivation [2,3] it actually may cause a profound suppression of REM sleep with a smaller decrease in non-REM sleep, which still occurs [2,7]. Thus it is really more a method of selective sleep deprivation. Analysis of graphic data on the use of hyperthermia suggests a similar effect [10]. Other approaches, such as continuous handling of the animal, require enormous amounts of labor. There is a need, then, for a relatively simple method of sleep deprivation which is supported by EEG data and which can process a number of animals at once with a minimum of labor. In this paper we shall describe such a technique, which consists of rats being kept awake by being placed in a slowly rotating cylinder.

METHOD

The apparatus is pictured in Fig. 1. Two cylindrical Nalgene tanks (68.6 x 35.6 cm) are placed horizontally on moving V belts. These are turned by a pulley system connected to a ¼ horsepower AC electric motor (Westinghouse KJ 75, type FHT) with a variable speed reducer (Zero-max, Model E, torque = 10 in. lb.). The cylinders rotate at a constant 3 RPM; the total distance covered by the rat is 4.8 km per 24 hr period. Inside the cylinder are a series of 5 dividers 35.6 cm in diameter connected by 7.6 cm squares of Plexiglas (Fig. 2). Thus each cylinder accommodates 6 rats. A 1.9 cm high strip of plexiglass tubing was glued to the floor of each of the six spaces in the cylinder, creating an obstruction over which the rats must walk. This made it less likely that the rat could merely slide along the moving surface of his compartment. The floor of each cylinder compartment is covered with a small amount of wood shavings which help absorb urine and provide a softer surface on which to walk. Each plexiglass divider contains a series of five 1 cm holes which allow circulation of air.

In order to document the state of consciousness of rats undergoing this procedure, we recorded the electroencephalogram and electromyogram of four 250 g Sprague-Dawley male rats who were in the moving cylinder for 24 hr, and compared them to seven animals in a stationary cage. During the 24 hour recording period, lights were on from 6:00 a.m. to 6:00 p.m. A detailed description of implantation of electrodes, recording apparatus, and criteria for analysis of the records has been provided in an earlier publication from this laboratory [8]. In order to introduce EEG cables into the rotating cylinders, a modified plexiglass divider system was used (Fig. 3). This particular system provides compartments for two rats; our present EEG data are taken from two 24-hr periods in which two rats each were recorded.

During 24-hr studies the cylinders were stopped twice for 15 minutes each. During these periods the animals were returned to their regular cages, in which food and water was available. While in their cages, they were stimulated manually if they appeared to be going to sleep.

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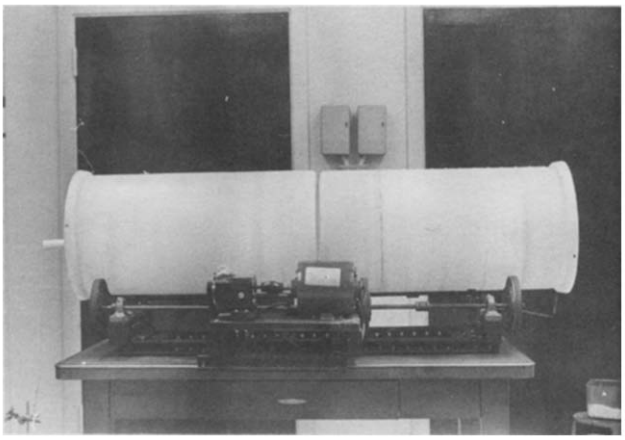

FIG. 1. Apparatus for sleep deprivation. The V belts rotate on the motor-driven pulleys, causing the cylinders to turn.

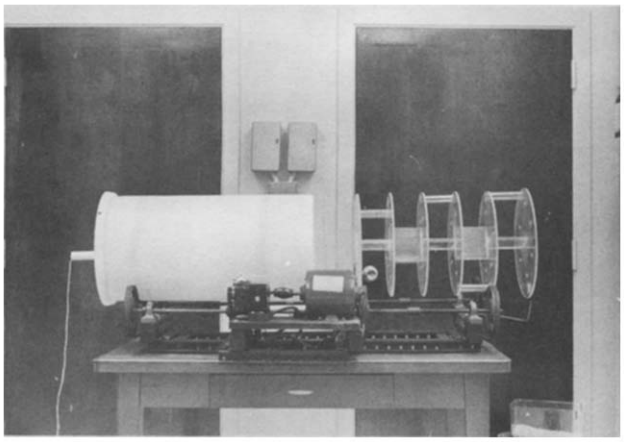

FIG. 2. Same apparatus as Fig.1, partially disassembled. The cylinder on the right has been removed, exposing the Plexiglas divider which separates each cylinder into six compartments which hold one rat each. The motor and speed reducer can be seen in the middle. The EEG cable protrudes from the cylinder on the left.

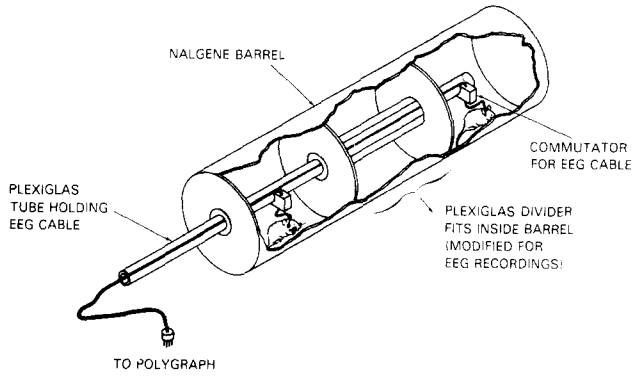

FIG. 3. Schematic drawing of modified Plexiglas divider used for EEG studies. A Plexiglas rod containing the EEG cables passes through the hollow center of the cylinder. At the end of each of the two horizontal cables is a commutator which allows the cables to hang downward and to rotate laterally with the rat's movement.

RESULTS

General

The rats tolerated the procedure well. There have been no deaths in rats who were sleep-deprived for 24 hr; no gross evidence of injury was found. There were no blisters on the footpads. An occasional rat in early studies was found to break one or more claws, and a resulting small hemorrhage was noted. This was greatly reduced after placing wood shavings in the cylinders. In one study, a thermistor was placed in one of the compartments, and temperature readings were taken periodically over the 24 hr. The maximum increase over baseline temperature was 2.2°C. In summary, the rats were maintained in a manner consistent with the standards of the National Institutes of Health for care of laboratory animals [8].

EEG

Data are presented in Table 1. The moving cylinder reduced total sleeping time from 47.0 percent of the 24 hr period to only 3.8 percent ($p<0.001$, two-tailed t-test). Similarly, REM and non-REM sleep (as a percentage of total recording time) were significantly decreased ($p<0.001$). The small amount of sleep which did occur was

TABLE 1

EEG ANALYSIS OF RATS IN SLEEP-DEPRIVATION APPARATUS FOR 24 HR*

	$\frac{\text{Total Sleep Time}}{\text{Total Recording Time}} \times 100$	$\frac{\text{REM Sleep Time}}{\text{Total Recording Time}} \times 100$	$\frac{\text{Non-REM Sleep Time}}{\text{Total Recording Time}} \times 100$
Control (n = 7)	47.0 ± 3.8	7.4 ± 0.5	39.6 ± 3.7
Experimental Group (n = 4)	3.8 ± 1.9	0.2 ± 0.0	3.6 ± 1.9
Significance†	$p<0.001$	$p<0.001$	$p<0.001$

*Values represent mean ± SEM.
†Two-tail t test.

made up of both REM and non-REM sleep; their percentages of total sleep time did not differ between the two conditions. Thus, there was no selective effect of one particular sleep stage, and instead total sleep time as a whole was markedly reduced.

DISCUSSION

We have presented a method of reducing total sleep to less than a tenth of normal for prolonged periods of time. Unlike the modified use of the platform surrounded by water, it does not totally suppress one sleep state (REM) while only partially suppressing another (non-REM sleep). A much larger number of rats (twelve) can be studied at one time than in previous procedures. It is anticipated that this will be useful in observing the effects of sleep deprivation in studies dealing with memory, neurochemistry, and possible circulating sleep factors.

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