Sleep: Effects of a Restricted Regime

Abstract. *Eight young male subjects were permitted to sleep only 3 hours out of each 24 for 8 days. Electroencephalographic recordings were made during the 3-hour period of sleep. There was an increase in the amount of deep sleep (stage 4) during this period. On a recovery night, the first 6 hours revealed a significant increase in deep sleep, and beyond this period there was a sharp increase in stage 1-rapid eye movement sleep.*

In the night-time sleep of adult humans, five "stages" of sleep can be reliably identified by the use of the electroencephalogram (EEG) and the electrooculogram (EOG). The stages are designated as 1, 1-REM (accompanied by rapid eye movements), 2, 3, and 4. (Below, we also speak of "stage 0." Actually, this consists of periods of wakefulness interspersed among periods of sleep.) It is reasonable to hypothesize that these various stages make different, or unequal, contributions in accomplishing the purpose or purposes of sleep. Although it would, of course, be quite useful to know the relative "value" of these stages of sleep, our present methodology does not permit a direct test of their relative efficiencies. The present experiment was designed to test indirectly the value of the various stages of sleep. In brief, it was decided that for 8 days the sleep of subjects would be restricted to a period of 3 hours out of 24. It was reasoned that the organism would selectively sleep in or "choose" those states which were dominant in the hierarchy of sleep need.

Eight healthy male students were selected as paid subjects for this experiment (mean age 17.6). Prior to beginning the experiment each subject slept for four uninterrupted nights in the laboratory while continuous base-line EEG and EOG recordings were being made. The procedures for all recordings in the experiment were identical with those used in a previous study (*1*).

On each of the experimental nights the subjects slept in the laboratory, where a continuous EEG and EOG were obtained during a 3-hour sleep period. Two pairs of subjects were studied each night. One pair slept from 2300 to 0200 and the other from 0230 to 0530. During the remaining 21 hours of the day the subjects were prevented from obtaining sleep. A system of monitors and checks was devised to assure that no subject napped during his waking period.

Performance tests were administered each night prior to the sleep period. A Paced Addition Test presented a pair of single digit numbers with a 2-second interval between each pair. The task was to add the digits, add eight to their sum, and write down the result. An X Test required the subject to listen to a taped recording of letters and press a signal button each time he heard *x*. A Pentagon Test displayed five lights, one of which was lighted each second. The task was to press a signal button each time a critical red light was flashed. These tests have been described in detail by Williams *et al.* (*2*).

The EEG records were scored for four stages of sleep minute-by-minute, with a modified version of the Dement-Kleitman scoring criteria (*1, 3*). In addition, the records were scored for stage 1-rapid eye movement sleep (1-REM).

Table 1 presents the basic data of the experiment. Three important effects of the restricted sleep regime appear in columns 2, 3, and 4 of this table. First, from a comparison of the base-line percentages for the full night's sleep with the percentages for the restricted 3 hours' sleep, it is clear that the limited sleep of the experimental nights was not simply a miniature of regular sleep. The *t*-tests for related measures revealed highly significant differences for all stages except stage 0 and stage 3.

Second, a comparison of the first 3 hours of base-line sleep with the 3-hour, restricted regime, by *t*-tests, showed that on the experimental nights there was a highly significant increase in the percentage of stage 4 sleep time and a significant decrease in the stage 3 percentage.

Third, in terms of absolute time, the subjects averaged some 86 minutes of sleep in stage 4 during the experimental nights. This is just short of the 92 minutes of this stage that would occur on the average during a 7½-hour sleep period, according to the base-line data. In contrast, the amount of time passed in the other stages of sleep during the experimental nights was sharply reduced in comparison with a full night's sleep. This is particularly true in the case of stage 2 and stage 1-REM. As to the latter, the subjects had an average of 13.5 minutes of stage 1-REM sleep during the experimental nights, compared with an average of some 104 minutes of such sleep during a normal 7½-hour period of sleep.

In order to determine whether there was an effect of the increasing length of time that the sleep of subjects was limited, a nights-by-subjects analysis of variance was performed for each sleep stage. There were no significant differences among the 7 nights for any of the stages. In a further examination of the stage 1-REM time across nights, it was noted that this stage was quite labile, varying in most subjects from zero or near zero to about 30 or 40 minutes of sleep. However, in one subject there did appear to be a consistent increase in 1-REM time as sleep deprivation progressed.

The length of sleep on the recovery night varied from 434 to 904 minutes. Data for this night are shown in the last three columns of Table 1. There were only minimal and insignificant differences between the percentages

Table 1. Mean percentage of total time spent in each stage of sleep on base-line, experimental, and recovery nights, and percentage of sleep in each stage during the first 180 minutes of base-line sleep, and during the first and the second 180 minutes of recovery sleep. The mean percentages were computed across the last three of the base-line nights and across seven experimental nights.

Sleep stage	Base line		Experimental nights	Recovery night		
	Full night's sleep	First 3 hours' sleep		First 3 hours' sleep	Second 3 hours' sleep	Full night's sleep
0	0.6	0.9	0.5	0.3	0.1	0.6
"1"*	5.2	4.2	2.5	2.4	7.5	5.2
1–REM	23.1	7.7	7.5	8.7	13.7	19.1
2	43.5	37.1	35.2	33.7	52.4	46.5
3	7.2	11.8	6.8	7.4	7.7	6.7
4	20.4	38.3	47.5	47.3	18.5	21.6

* This is the stage 1 which remains after subtracting the 1-REM time.

for the full recovery night's sleep and those for the full base-line nights. However, a comparison of the first 6 hours (analyzed as two 3-hour periods) of the recovery night with comparable base-line sleep revealed marked differences. The first 3 hours of sleep on the recovery night were similar to the 3 hours' sleep of the *experimental* nights (*4*). Compared with the first 3 hours' sleep on the base-line nights, in the first 3 hours of recovery sleep there was an elevation in the amount of stage 4 sleep, a decrease in the percentage of stage 3, and no significant differences in the percentage of the other stages. During the second 3 hours' sleep of the recovery night, when compared with the second 3 hours of base-line sleep (not shown in the table), there was a continued significant percentage elevation of the stage 4 sleep, and a significant depression of the stage 1-REM sleep.

The stage 4 and stage 1-REM characteristics during the recovery night were further examined. It was noted that 83 percent of the total stage 4 sleep obtained during the recovery night occurred during the first 6 hours of sleep. In contrast, 69 percent of the 1-REM sleep occurred *after* these first 6 hours. Indeed, it was found that the amount of stage 1-REM obtained was almost a direct function of the time slept beyond 6 hours. It is tempting to infer that the length of "oversleep" was determined by the "need" for stage 1-REM sleep.

There was evidence from the behavioral tasks that performance had begun to deteriorate on the seventh and eighth nights of the testing periods. Sixty-one percent of the errors on the X task, and 41 percent of the errors on Pentagon task, occurred on nights 7 and 8. Because of the learning which occurred during testing on the addition task, performance on this test on nights 7 and 8 was compared with a subject's best performance night (presumed to be the asymptote of their learning within the experiment). There was significantly poorer performance as evaluated by a *t*-test of related measures with a one-tail test. The mean numbers of errors or omissions for the best night and the 7th and 8th nights, respectively, were 32.2, 42.4, and 43.9.

These decrements were neither uniform nor fully consistent. On the X task: four of the eight subjects made no errors, or only one, on night 7; three had zero or only one error on night 8; and five subjects had zero or one error on either night 7 or 8. On the Pentagon task five subjects were errorless on night 7, and six on night 8; seven had no errors on either night 7 or 8. Similarly, on the addition task three subjects equaled or gave their best performance on night 7 and two did so on night 8. The best performance of four subjects was either on night 7 or 8.

It has previously been shown that there is a differential patterning of the proportions of the various sleep stages which occur during successive periods during a full night of sleep (*1*). A high proportion of the stage 4 occurs during the first third of the night's sleep. Conversely, stage 1-REM sleep predominates in the final third of sleep. During the restricted 3-hour sleep periods, our subjects continued to pattern their sleep much as they did during the first 3 hours of base-line sleep, except for an increase in stage 4, largely at the expense of stage 3. The restriction of sleep to 3 hours, and the increased percentage of that time that was devoted to stage 4 sleep, naturally resulted in severe deprivation of sleep in stages other than stage 4. This was particularly true of stage 2 (absolutely) and stage 1-REM (absolutely and relatively). Whether further restriction of total sleep, and the attendant deprivation of various stages of sleep, would finally modify this pattern of sleep is not known, but evidence for this notion occurred with one subject.

The strength of the stage 4 sleep response may attest to its "value" in the sleep response hierarchy, or it may simply reflect the stability of sleep patterning. A previous study of stage 4 deprivation demonstrated a "need-like" character of this stage of sleep (*5*). Further, in a study of sleep deprivation, Berger and Oswald found that even though the pressure for 1-REM sleep mounts during deprivation, the stage 4 requirement is even greater and takes priority during the first night of recovery (*6*). These results strongly suggest that in the rivalry between stage 4 and 1-REM the former tends to displace the latter in the first part of the sleep period.

The early part of the recovery night was "dominated" by stage 4 sleep (47.3 percent), even though this stage suffered the least deprivation during the restricted sleep regime. The delay of the appearance of substantial amounts of stage 1-REM sleep until the stage 4 sleep had run its course during the recovery night supports increasing evidence that high-voltage, slow-wave sleep (stage 4), besides serving as an inhibitor of the reticular activating system (*7*), also serves as a release mechanism for the limbic midbrain system, which has been shown by Jouvet (*8*) to be related to the low-voltage, high-frequency sleep which is characteristic of stage 1-REM. It is a common observation in human subjects that a substantial amount of stage 4 (high-voltage, slow-wave) sleep is propaedeutic to the appearance of an uninterrupted sequence of stage 1-REM. In the rat it has been found that 96 percent of 121 recorded episodes of low-voltage, fast-wave sleep were preceded by more than 6 minutes of high-voltage, slow-wave sleep (*9*), and all episodes were preceded by at least 3 minutes of higher voltage slow waves. Further, Sovrad and Kamarova (*10*), have shown that rats injected with bulbocapnine (which suppresses the amplitude of the high-voltage slow waves) show no episodes of low-voltage, fast-wave sleep until the amplitude of high-voltage sleep waves has returned to the base-line level.

We cannot say whether the performance decrements stemmed from a decrease in motivation (although the subjects said they were trying to give maximal performances), or from an inability to respond efficiently. It is important to note, however, that this decrement occurred even though the subjects had undergone only a very limited amount of deprivation of stage 4 sleep. Whatever the basis, it may be said that a chronic, partial deprivation of sleep does result in a response decrement.

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11. We will supply the individual subject data and the performance test data upon request. We thank R. K. Weaver and Joseph Pylka for their assistance in this project. Supported by Air Force Office of Research grant AF-AFOSR-395-65.

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Humoral Mediation of Radiation-Induced Motivation in Parabiont Rats

Abstract. *Parabiont rat pairs with a skin–vascular anastomosis were used to test for a radiation-induced transferable humoral factor which would motivate aversive conditioning in a nonirradiated (shielded) recipient. The shielded partner, by tasting saccharin-flavored fluid shortly after its partner was x-irradiated (360 roentgens), was aversively conditioned to saccharin. This was interpreted as evidence for a humoral motivating factor.*

Exposure to ionizing radiation has been used as a motivating stimulus in the conditioning of aversive reactions; this noxious effect has been demonstrated in several species and in a variety of learning situations (*1*, *2*). An aversion to drinking saccharin-flavored water has been obtained in rats by pairing their tasting of saccharin (the conditioned stimulus) with a single irradiation. There are indications that radiation-induced motivation may be initiated by non-neural (humoral) consequences of exposure rather than by prompt neural stimulation. Aversive conditioning has been obtained when the saccharin was presented during irradiation at the rate of 0.7 mr/sec (*1*), which is much lower than the rates that have been used thus far in eliciting prompt reactions (*2*). A conditioned saccharin aversion has also been obtained by presenting the saccharin within the first few hours after exposure at higher rates (*3–5*). Such conditioning after exposure does not depend on trace effects of prompt neural stimulation since conditioning has been obtained with this arrangement in rats that were deeply anesthetized (ethyl ether) during their irradiation (*6*).

Our investigation was designed to test for humoral mediation of radiation-induced motivation. Unrestrained parabiont rat pairs, united by vascular anastomosis, were used to determine whether irradiation of one partner would produce a transferable systemic humoral factor which would motivate the conditioning of a saccharin aversion in the nonirradiated (shielded) partner.

Male littermates of the specific pathogen-free strain of Sprague-Dawley rats bred at the U.S. Naval Radiological Defense Laboratory were paired by weight on removal from their litter at 24 ± 3 days of age and the parabiosis operation was usually performed 1 to 2 days later. A skin-to-skin union, forming a common lateral skin flap, was made with the Bunster-Meyer technique (*7*), modified by deleting the abdominal muscle union. The skin suture clips were removed 7 to 9 days after the operation. Losses among these animals resulted primarily from parabiosis intoxication (*8*, *9*) and, much less frequently, from infection, physical separation, or chronic fighting. These losses amounted to 43 percent and always occurred within 3 weeks after the operation. The remaining pairs were subjected to the irradiation-conditioning procedure at 37 to 44 days of age. Within 24 hours after completion of the conditioning test, patency of the vascular anastomosis was tested in each pair by counting the activity in blood samples from both partners 1 hour after injection of Fe^{59}-labeled red cells into the irradiated partner. To serve as physical and behavioral controls approximating the parabiont condition without anastomosis, additional animals were physically tied in pairs by joining lateral skin folds with wound clips. Losses among tied pairs arose from tie separation and, sometimes, from chronic fighting. These losses amounted to 31 percent.

Separate measurement of drinking behavior for each partner was provided by a twin-tunnel device leading from the living cage to the drinking spouts (Fig. 1). For a period of 4 to 5 days prior to the start of experimental treatment the animal pairs were habituated cooperatively to use the tunnels in drinking.

A double-chambered radiation exposure unit was used to provide nearly complete shielding of the test partner of each pair. One member of each pair, randomly selected as the right- or left-hand partner, was exposed to 360 r with the x-ray machine operated at 250 kv (peak) and 15 ma (half-value layer, 1.5 mm Cu). The exposure rate, measured in air within the

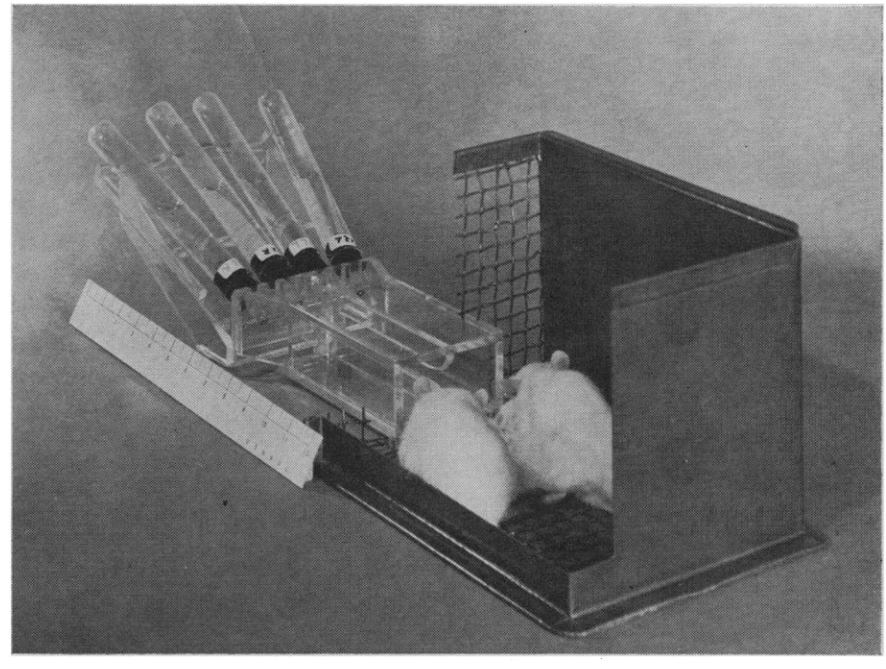

Fig. 1. Twin tunnels used to provide a separate drinking station for each partner of conjoined rat pairs. Shown in position on living cage (cut-away view). The septum between tunnels is slotted to accommodate the skin flap that unites the partners. The length of the tunnels suffices to preclude drinking at the incorrect station. Two drinking tubes, as used in the saccharin preference test, are shown at each station, and center positions are available for single-tube presentations. One-foot (30.5 cm) scale shown.