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Sleep Deprivation in the Rat: X. Integration and Discussion of the Findings

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Summary: The results of a series of studies on total and selective sleep deprivation in the rat are integrated and discussed. These studies showed that total sleep deprivation, paradoxical sleep deprivation, and disruption and/or deprivation of non-rapid eye movement (NREM) sleep produced a reliable syndrome that included death, debilitated appearance, skin lesions, increased food intake, weight loss, increased energy expenditure, decreased body temperature during the late stages of deprivation, increased plasma norepinephrine, and decreased plasma thyroxine. The significance of this syndrome for the function of sleep is not entirely clear, but several changes suggested that sleep may be necessary for effective thermoregulation. **Key Words:** Sleep deprivation—Function of sleep—Energy expenditure—Thermoregulation.

This article summarizes the first nine reports in this series on sleep deprivation in the rat (1–9), reviews relationships among the separate studies, and discusses issues common to them. Salient features of these studies were the maintenance of deprivation long enough to reveal serious physiological effects and the use of controls for the stimulation that prevented sleep or selected sleep stages (1). Controls were achieved by simultaneously housing an experimental rat and a control rat on two sides of a divided horizontal disk suspended over water. Whenever the experimental rat started to sleep or entered a "forbidden" sleep stage, the disk was automatically rotated, which awakened the experimental rat and forced both rats to walk opposite to disk rotation to avoid the water. Thus, both rats received the same physical stimulation, but the stimulation was timed to awaken the experimental rat. Although the control rat was also awakened if it was asleep when the disk was rotated, it could sleep ad lib when the experimental rat was spontaneously awake (2).

SUMMARY OF RESULTS

All rats subjected to chronic total sleep deprivation (TSD) (3) or chronic paradoxical sleep deprivation (PSD) (4) showed the following sleep deprivation effects (SDEs):

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1. Mortality: Deprived rats died or were sacrificed when death seemed imminent. Survival was 11 to 32 days in TSD rats (n = 9) and 16 to 54 days in PSD rats (n = 12). At least for rats, sleep and paradoxical sleep (PS) serve vital functions that are relatively well protected.

2. A progressively debilitated, scrawny appearance with brownish, disheveled fur: This appearance change was not simply a result of terminal processes, since it began well before death and is not an inevitable correlate of death in rats.

3. Severe ulcerative and hyperkeratotic skin lesions localized to the tails and the plantar surfaces of the paws and the tails (6): The pathogenesis of the lesions was not resolved.

4. Increased food intake: Group means for the final quarter of survival reached more than 80 and 100% above baseline levels in TSD and PSD respectively.

5. Weight loss: Group means decreased 17.9 and 21.6% from baseline weights in TSD and PSD rats respectively. The loss of weight, in spite of increased food intake, could not be explained by dehydration, malabsorption, or gross perturbations of intermediary metabolism. The weight loss alone was not sufficient to explain the deaths of the sleep deprived rats.

6. An increase in energy expenditure (EE), which was calculated from the caloric values of food intake, weight change, and wastes, and was confirmed by indirect calorimetry (5): Mean EE during the final quarter of survival was more than twice baseline levels. It could not be fully explained by the metabolic cost of increased wakefulness, motor activity, or water exposure. An increase in resting EE was indicated and supported by an increase in heart rate during NREM sleep in PSD rats. The mediation of the increased EE has not been resolved.

7. A decrease in body temperature (T_B) beginning near the middle of the survival period (except for one TSD rat that showed a precipitous one day decline in T_B just prior to sacrifice). In TSD rats, the T_B decline was preceded by an increase in T_B during the first half of survival.

8. An increase in plasma norepinephrine (NE) was found in deprived rats.

9. A decrease in plasma thyroxine (T_4) and an increase in the ratio of plasma triiodothyronine (T_3) to T_4 (T_3/T_4 ratio) was also found in deprived rats.

Effects 1 through 6 were also observed in all rats deprived of high EEG amplitude NREM sleep (HS2) (8). HS2 deprived (HS2D) rats did not show a large T_B decline until just prior to death. Effects 8 and 9 could not be evaluated in these rats, because blood samples were not taken. It was not resolved whether the SDEs in HS2D rats resulted from loss of HS2 or from the fragmentation of NREM sleep that was intrinsic to the deprivation procedure.

Where the appropriate variables were measured, the SDEs were also seen in eight rats that were totally or selectively sleep deprived to the point of severe pathology before they were allowed to sleep ad lib (9).

No yoked control (TSC, PSC, and HS2C) rat died or reached the point where death seemed imminent. On all variables listed above, changes in sleep-deprived rats were greater than changes in yoked control rats. On most variables, TSC and HS2C rats changed from baseline in the same direction as the respective sleep-deprived rats. These changes could have resulted from partial sleep loss in the control rats. Similar changes from baseline occurred less frequently and to a lesser degree in PSC rats, possibly because their sleep was little changed from baseline. Alternatively, it could be reasoned that PSC rats underwent fewer disk rotations than TSC and HS2C rats. To

choose between these two alternatives in TSC rats, we computed the correlations between TS loss, PS loss, and percentage of time the disk was rotated versus the percentage change from baseline during the deprivation period of five outcome variables on which these rats showed substantial monotonic or near monotonic changes in the same direction as the TSD rats: EE, heart rate, epinephrine, NE, and T_4. Probably as a result of restricted variance, none of the 15 correlations were statistically significant. However, it was noteworthy that disk rotation correlated positively with change in only one of the outcome variables (epinephrine), whereas percentage PS loss from baseline correlated positively with all five outcome variables. Total sleep (TS) loss correlated positively with only two of the outcome variables, but individual differences in TS loss were relatively small in TSC rats; the coefficient of variation was 0.021 for TS loss compared to 0.046 for PS loss. In HS2C rats, EE increase showed a small positive correlation (0.20) with PS loss, but a large negative correlation (-0.60) with disk rotation. The combined data suggest that disk rotation was not important in producing SDE-like trends in control rats, but that partial PS loss may have been a contributing variable.

The SDEs reflect a specific syndrome, rather than generalized impairment of all physiological systems. Except for data obtained just prior to death in some variables, there were no significant differences in TSD and PSD rats versus their respective controls in the following: plasma levels of sodium, potassium, T_3, and adrenocorticotropic hormone (ACTH); urinary urobilinogen, nitrite, bilirubin, glucose, ketone, specific gravity, pH, or protein (3,4); rates of hair growth, mitotic index in the jejunum (6); in vivo and in vitro tests of immune function (7). A previous study (10) revealed no histological abnormalities (at the light microscope level) in brains and other major organs. PSD rats (4) showed no histological abnormalities in glands and viscera.

Although survival time varied considerably across rats, death did not occur unpredictably. Survival time was negatively correlated with the rate of EE increase (slope of regression line) during the first half of survival; for TSD rats, $r = -0.81$, $p < 0.01$; for PSD rats, $r = -0.63$, $p < 0.05$; for HS2D rats, $r = -0.90$, $p < 0.01$. Thus, survival could be predicted from a specific SDE that occurred well before the sleep-deprived rats were extremely debilitated or near death. Other measures were not tested as predictor variables, either because they were used as sacrifice criteria (T_B), could not be quantified precisely (lesions), did not change greatly early in deprivation (appearance, NE, T_4), or were already included in EE (food and weight). Apparently, variability in survival time was in part determined by individual differences in the rate of SDE development. This conclusion also follows from the relatively small individual differences in major SDEs (compared to the large individual differences in survival) when rates were equated by calculating means by quarters of survival.

The proximal cause of death in sleep-deprived rats was not determined. Morphological abnormalities that might have caused death were not identified in necropsy or histological examinations. Hypothermia is a candidate, but the low preterminal T_B could have been an effect rather than a cause of dying. However, the start of the T_B decline near the middle of the deprivation period occurred too long in advance of death and while the animals were still too healthy to be explained as a terminal process. To understand the pathological processes that eventuated in death, it could be helpful to know the sequence of SDEs. However, group data can distort sequences in individual animals, and, within individual animals, it was often difficult to distinguish the start of an experimental effect from baseline variation. The only conclusion our judgement

permitted with confidence was that EE and heart rate increased early, whereas other SDEs tended to appear later.

RECOVERY STUDIES

Eight rats (five TSD, two PSD, and one HS2D) were allowed to sleep ad lib after they showed major SDEs (9). The three rats that had shown the largest T_B declines (one PSD and two TSD rats) died within a few days. The other five rats recovered from all SDEs. EE, T_B, and NE started to recover on the first recovery day. EE returned to baseline levels or lower within one to three days. In all surviving rats, recovery sleep featured huge, immediate PS rebounds and inconsistent or relatively small rebounds of other sleep stages. The priority for PS following chronic sleep deprivation was emphasized by sleep onset PS or short latency to PS during all deprivation conditions.

POTENTIAL CONFOUNDS

In the introduction to this series (1), we emphasized that sleep loss could be reasonably interpreted as the cause of subsequent effects only if other consequences of the experimental stimuli were not plausible mediators of these effects. The effects of physical environment are ruled out, because the environment was the same for sleep-deprived and yoked control rats. The effects of physical stimulation are ruled out, because physical stimulation was not only benign, but also the same for deprived and control rats. Exercise was sometimes greater for deprived rats, sometimes greater for yoked controls, but at no time was it very great for either. We estimate that TSD rats and their controls, which were subjected to the most disk rotation, were forced to walk an average of less than a mile a day. When provided with a running wheel, rats may voluntarily run 30 miles a day without apparent ill effects (11). Control studies indicated that the SDEs did not result from water exposure.

Stress

One remaining issue of consequence is whether stress responses may have mediated the SDEs. The issue may be separated into two questions. (a) Were the sleep-deprived rats more stressed than their yoked controls? (b) Did the SDEs result from stress? We think that only the second question is critical for the major issue of whether the SDEs did indeed result from sleep loss. However, we will answer the first question as well, because it has been asked frequently in discussions of our results. Generally, the kind of information requested has been about the classical indicators of stress (12).

Of the classical indicators, the strongest evidence for stress in the sleep-deprived rats was adrenal hypertrophy. Relative to their respective yoked controls, adrenal weights in rats carried to death or imminent death were 59.0% greater in TSD rats, 48.8% greater in PSD rats, and 45.7% greater in HS2D rats. However, it is likely that much of the increase occurred during the stress of dying, since adrenal hypertrophy can occur between 6 and 24 h following the imposition of a severe and continued stressor (13,14). In four PSD rats that were sacrificed after SDEs (EE increase, skin lesions, debilitated appearance) were readily apparent but before death appeared imminent, adrenal weights were only 22.2% greater than in controls. For two TSD rats also sacrificed after SDEs were apparent but before death was imminent, mean adrenal weight was slightly lower than in controls.

In our first TSD study (15), we reported that three of eight TSD rats showed stomach ulcers. An examination of the affected tissue by two professional pathologists yielded the opinion that these were not ulcers but mucosal erosions that could have resulted from autodigestion during the death process. Most TSD, PSD, and HS2D rats developed similar pinpoint erosions, but none showed large, crater-like, perforating, or hemorrhaging ulcers of the stomach lining such as those that have been reported in stressed rats (16,17) or which we saw in all food-deprived rats. The absence of ulcers in the sleep-deprived rats was not too surprising, since they increased their food intake. In rats, ulcer formation under stress is largely dependent upon reduced food intake (17,18).

In neither the TSD nor PSD experiments was there a significant group × time interaction for ACTH. There was a tendency for higher ACTH levels in deprived rats, but mostly in the last half of deprivation, after SDEs were already apparent. Corticosteroids were significantly elevated only in the TSD rats; again, the increases over control levels were not apparent until the second half of deprivation. Therefore, it is more likely that these stress responses were caused by or independent of the SDEs rather than the cause of them. The maximal mean quarterly increase in corticosteroids was approximately 75% over baseline, which was less than typically reported in stress studies. Long-term stress by water restriction or cold exposure produced 100 to 200% increases (19). Short-term stress by foot shock or confinement (20) and by transfer to an unfamiliar environment (21) have increased corticosteroids to levels 5 to 10 times those reported here. Furthermore, in three TSD rats in the recovery study (9), corticosteroids decreased during deprivation. Given the severity and multiplicity of pathology in sleep-deprived rats, their classical stress symptoms were very mild.

If classical physiological stress symptoms were not prominent in the sleep-deprived rats, it probably means that these symptoms poorly reflect some stressful states, because some internal condition of stress is a reasonable inference from the rats' pathology—if not from sleep loss itself. However, the presence of stress in this sense does not necessarily imply that it caused the SDEs. If one automatically reached that conclusion, one would have difficulty in verifying that almost any pathological condition, such as severe hunger or thirst, had biological significance apart from stress. Almost any threat to the biological integrity of the organism is likely to be stressful in one sense of the word or another. Stress is a useful concept for describing a set of nonspecific emergency reactions to a wide variety of threatening conditions. Whether there is a substantive threat apart from the emergency reactions must be decided by the presence or absence of specific consequences that are not part of the nonspecific stress reactions, e.g., malnutrition as a consequence of food deprivation and dehydration as a consequence of water deprivation. Therefore, the critical question is whether the SDEs are simply part of a nonspecific stress response.

The answer is an unambiguous "no." All deprived rats showed a specific syndrome: a large increase in food intake; weight loss; debilitated appearance; severe ulcerative and keratotic lesions localized to the tails and plantar surfaces; when measured, increased plasma NE, decreased plasma thyroxine, and an increase in the T_3/T_4 ratio; eventual death or impending death. In addition, most sleep deprived rats showed an eventual decline in T_B in spite of markedly increased EE. We know of no stress or punishment paradigm, including the executive monkey experiment (22), which produces this specific syndrome. Therefore, the weight of evidence on the second, and important stress question is that the SDEs did not result from stress.

Reduction of circadian rhythms

Since our rats were run in constant light, it has been suggested by some colleagues that the SDEs could have resulted from the consequent reduction of circadian rhythms. This possibility is highly unlikely. Our rats did not show SDEs when they were maintained in constant light prior to deprivation; yoked control rats did not show SDEs of comparable severity; we know of no reports of similar SDEs in rats whose circadian rhythms have been reduced or eliminated by constant light, constant dark, or lesions of the suprachiasmatic nucleus. At most, disruption of circadian rhythms would have had to produce SDEs via an interaction with sleep loss. However, even this possibility seems unlikely, since, as shown in the literature review to be presented below, some sleep deprivation studies carried out with normal light–dark rhythms have produced similar SDEs.

Ineffective grooming

Colleagues have suggested that, although time spent grooming was similar in sleep deprived and control rats (3,4), ineffective grooming in deprived rats could have caused the poor condition of the fur, which in turn decreased its insulating properties and produced some of the observed declines in T_B. We cannot rule out ineffective grooming as a source of poor fur condition. However, it seems an unlikely source of the T_B decline, because, during recovery from sleep deprivation, T_B and EE recovered several days in advance of the recovery of the fur (9).

Apparatus effects

It is unlikely that the SDEs were specific to the apparatus we used. As will be shown in the literature review to be presented later, other studies using different deprivation procedures have produced similar, albeit generally less severe, SDEs. We did experiment with another apparatus similar to the lower half of an activity wheel. When the rat entered a "forbidden" sleep stage, the wheel was partially rotated, thereby carrying the rat toward a vertical position and forcing it to awaken and walk to the level portion of the wheel to avoid falling off. This apparatus was not completely successful because, as deprivation progressed, the rats sometimes failed to awaken even when they fell from the side of the wheel. It also put too much stress on the recording cable, which eventually caused the recording plug cemented to the rat's head to loosen or dislodge. We were, however, successful in maintaining one rat on PSD for 41 days. Food intake increased progressively, weight declined progressively, and EE increased to approximately 60% above baseline levels. When ad lib sleep was permitted, EE returned to baseline within a few days.

RELEVANCE TO THEORIES OF SLEEP FUNCTION

The present results show that, at least in the rat, chronic total or selective sleep loss produces reliable physiological effects. To understand the underlying functions, we need to know (a) which effects are primary, i.e., which occur first and cause the others; (b) how the primary effects are mediated, i.e., what "goes wrong" to produce them;

and (c) how sleep prevents this mediation. Hopefully, such knowledge will eventually form a coherent matrix with results of stimulation and correlational studies.

Since sleep and its stages may have multiple functions, the present results do not rule out any theory of what the function of sleep is; they do rule out some theories of what the function is not. For the rat at least, the major systemic effects of sleep loss rule out claims that sleep is *only* for behavioral adaptation or higher mental processes. Eventually, comprehensive theories of sleep function will have to explain the SDEs. More immediately, researchers will have to control for the SDEs in testing for other effects, e.g., performance changes that might be affected by hunger.

THE ROLE OF THE BRAIN

Brain activity changes with sleep and wakefulness, and changes in brain activity may mediate the effects of sleep loss, but this does not necessarily mean that the brain itself is a primary functional target of sleep. Except for preterminal electroencephalogram (EEG) amplitude decline and signs of motor dysfunction, which might be expected near death from any cause, there is as yet no definite evidence that brain function was impaired by our deprivation procedures. Eventually, it will be necessary to resolve the role of the brain in mediating sleep function and resolve whether the brain itself is a functional target of sleep. The reliable systemic effects of chronic sleep loss identified in the current studies could be clues to what to look for in the brain.

FUNCTIONAL ROLES OF INDIVIDUAL SLEEP STAGES

The functional roles of individual sleep stages are difficult to establish in any absolute fashion, because no stage deprivation was completely selective. Furthermore, even if stage deprivation were completely selective and specific deficits followed, it would only show that the stage was necessary for the implied function, not that it was sufficient. The function might be served only by the normal interaction of the stage with other stages. It is with these caveats in mind that we proceed with the discussions below on the contributions of specific stages to SDEs in general and to thermoregulatory deficits more specifically.

Since PSD was confounded with partial HS2 loss, it is possible that PSD caused SDEs only in combination with HS2 loss. However, the large, immediate PS rebound priority during recovery from all deprivation procedures, the rapid reversal of several SDEs during or following this rebound, the absence of early HS2 rebounds, the short latency to PS in the TSD and HS2D procedures—in combination with the PSD results—strongly suggest that PS is essential to prevent most of the observed SDEs. The major exception is the rise in T_B early in HS2D when PS was still present.

The TSD study provided no evidence on whether NREM is essential, because NREM and PS loss were completely confounded. At the very least, however, NREM has a functional role related to that of PS, since, with the exception of the late T_B decline, SDEs were accelerated in TSD compared to PSD. The HS2D study provided some evidence for an essential NREM role, since control studies showed that the PS loss suffered by HS2D rats was insufficient to produce serious SDEs. It is possible that the HS2 loss (or NREM interruption) might not have caused SDEs in the absence of a substantial PS loss. But even this alternative would mean that either HS2 or NREM continuity or both are essential for preventing SDEs at least when PS levels are low.

To say that NREM has a "functional role" in preventing or retarding SDEs blankets

much ignorance about the nature of this role. Do NREM and PS additively fulfill one sleep function? If so, why don't PSD rats increase their NREM during deprivation, and why are NREM rebounds during early recovery either weak or absent? Does NREM serve as a primer, substrate, or stimulus for PS? If so, why should rats normally have about six times more NREM than PS, whereas TSD rats have sleep onset PS and virtually no NREM prior to the large PS rebounds of recovery? In addition to sharing a functional target with PS, does NREM have a functional target of its own? If so, we might again ask why NREM rebounds were not prominent in early recovery from TSD. It may not be possible to resolve the issues of rebound priority until specific functions are identified.

ENERGY AND TEMPERATURE REGULATION

Although the present results do not reveal the function of sleep, the early and eventually large increases in EE suggest energy regulation as one major function. Large increases in EE could result from either abnormal calorigenic mechanisms or from a normal calorigenic response to an abnormally high energy need.

Considering abnormal mechanisms, it is unlikely that the increased EE was caused simply by an increase in circulating thyroid hormones or epinephrine; increased circulating NE could have played a role in increasing EE. Sleep deprivation could have produced other calorigenic changes in the thyroid system (e.g., increased thyroid turnover) and sympathetic system (activation of brown adipose tissue). Additional possibilities that have not been explored include increased receptor sensitivity for calorigenic hormones, uncoupling of oxidative phosphorylation, and perturbations of yet unidentified calorigenic mechanisms.

Recent results from our laboratory favor an increase in the need for energy over abnormal calorigenic mechanisms as the explanation for the increase in EE. As indicated earlier, of the putative hormonal mediators of the increased EE that we evaluated, NE was the most likely candidate. Therefore, we sought to determine the effects of TSD in rats whose increase in NE was blunted by the administration of guanethidine, a postganglionic sympathetic blocker (23). These rats showed very large increases in EE, and much larger increases in circulating epinephrine compared to the original TSD rats. Apparently, epinephrine substituted for NE as a calorigenic stimulus. These results suggest that, rather than stimulating uncontrolled calorigenesis, TSD produces an abnormally high need for EE, which is met by whatever mechanisms are available.

The need for energy was increased somewhat by the metabolic cost of increased wakefulness and, in some rats, by motor activity, but not by nearly enough to explain the magnitude of the EE increase. A failure of thermoregulation could have increased the need for energy.

Our data suggest two specific thermoregulatory deficits that could increase the need for EE: (a) excessive heat loss, which is most likely attributable to the loss of PS and which would increase the need for EE to maintain T_B near normal levels; and (b) an increase in preferred T_B or "setpoint," which is most likely attributable to the loss or disruption of NREM sleep and which would increase the need for EE to sustain higher than normal temperatures.

The heat loss hypothesis derives from the negative correlation between EE and T_B (EE rose as T_B fell) during the last half of deprivation in TSD, PSD, and HS2D. According to this hypothesis (a) increased EE initially generated sufficient heat to

compensate for excessive heat loss; and (b) when heat production could no longer match heat loss, T_B declined. This hypothesis is supported by a hormonal profile in both PSD and TSD rats similar to that in cold stress (5).

Excessive heat loss is hypothetically attributed to the loss of PS. T_B declined in PSD rats, which lost very little NREM. HS2D rats did not show a substantial decrease below baseline T_B levels until the last decile of survival (8), when PS% had dropped from a prior deprivation mean of 2.55% to a mean of 0.46%. Early recovery from TSD, PSD, and HS2D was characterized by increases in T_B and large amounts of PS (9).[1]

Since TSD rats suffered virtually complete loss of PS, it may be asked why the mean T_B decline from baseline was approximately three times as great in PSD rats as in TSD rats (5). The T_B decline may have been greater in PSD rats because, as a result of chronologically longer survival times, they had a greater accumulated PS loss.

Given the early rise in T_B and EE in TSD rats, the assumption that they underwent the same excessive heat loss process as PSD rats leads to the conclusion that the TSD rats had an elevated setpoint: The TSD rats would not be expected to expend additional energy to maintain T_B unless T_B was falling below the setpoint level. The only way T_B could be elevated and still be below setpoint is if the setpoint itself were raised. This elevation of setpoint is attributed to some aspect of NREM loss or disruption, because T_B was elevated by about 0.5°C during portions of TSD and HS2D (5.8) but never rose appreciably above baseline during PSD, thus suggesting that PS loss was insufficient to raise the setpoint. The lack of a rise during PSD also argues against an apparatus effect, suggesting that T_B rises in HS2C and TSC rats resulted from NREM disruption.

Some confirmation for these hypotheses is found in recent data from our laboratory (24). TSD rats were shown to prefer progressively elevated ambient temperatures (and thus higher T_Bs), even when they had above-baseline T_Bs. In addition to confirming an elevated setpoint, these data support the hypothesis of excessive heat loss, since the rats were unable to achieve the elevated setpoint in spite of greatly increased EE.

Since TSD rats suffered both PS and NREM loss, the corresponding deficits should push T_B in opposite directions, so that T_B in TSD cannot be predicted from the hypothesized deficits. Post hoc, it could be assumed that in TSD the raised setpoint induced by NREM loss had an initially greater effect on T_B, whereas the heat loss induced by PS loss had a subsequently greater effect. The assumption is not entirely gratuitous. T_B was not depressed below baseline levels until the second half of PSD. As reviewed previously (8), studies with relatively short-term TSD generally show a rebound priority for high-amplitude, slow-wave NREM sleep, whereas our study of chronic TSD showed a decided PS rebound priority.

The two hypotheses described above suggest that sleep is necessary for effective thermoregulation. Major questions remain unanswered: (a) Will the model be confirmed by other measures of thermoregulatory mechanisms, e.g., thermal resistance, response to thermal challenge? The parameters we measured were not originally di-

[1]The heat loss hypothesis returns attention to the effect of water immersion in sleep-deprived rats, since heat loss could be increased by conduction while the rats are in the water and by evaporative cooling when they emerge from the water. We reported earlier (5) that control immersions did not appreciably increase daily EE, which makes it unlikely that immersions per se appreciably affected daily heat loss. However, the effect of control immersions on T_B was not evaluated. Nevertheless, it is highly unlikely that immersion was a primary factor in altering T_B, since TSD rats had above baseline T_Bs during most of deprivation and smaller T_B declines than PSD rats at the end of deprivation in spite of having greater rates of water immersion and longer times in the water (3,4).

rected to thermoregulation, because we did not start with a thermoregulatory model. We had to make several inferences about thermoregulation from other data. (b) Are there other sleep deprivation-induced thermoregulatory deficits we have not identified? (c) Which thermoregulatory mechanisms are impaired? Are they central, peripheral, or both? (d) Parmeggiani (25) and Glotzbach and Heller (26) have shown that autonomic thermoregulation is suspended during PS. Why then should PS be necessary to prevent excessive heat loss? Is the suspension necessary to allow the thermoregulatory system to readjust or recuperate? (e) Can adjustment of thermoregulation be used to explain other aspects of sleep physiology and behavior? (f) Why should a state so complicated and time-consuming as sleep be necessary for effective thermoregulation? (g) Do the thermoregulatory deficits that follow sleep loss reflect primary functions of sleep, or are they secondary to other functions, e.g., maintenance of a neurochemical or organ system? (h) Are the putative thermoregulatory functions of sleep simply one part of more generalized regulatory functions?

One theory that linked thermoregulation and sleep (27) emphasized the energy savings during sleep that result from its lower T_B. The data from sleep-deprived rats suggest that the role of sleep in thermoregulation extends beyond energy savings during sleep to maintenance of effective long-term thermoregulation in all states.

The thermoregulatory model we have described has some potential for explaining ontogenetic and phylogenetic variations in sleep. Infant mammals and small mammals, which, because of their larger surface to mass ratio, presumably have more difficulty thermoregulating, sleep more often and more of the time than mature and larger mammals (28,29). Reptiles show behavioral and electrophysiological patterns comparable to those of mammalian NREM sleep, as well as comparable responses of these patterns to hypnotic and analeptic drugs (30), but they do not show PS. Perhaps reptiles need NREM sleep because it is important for establishing setpoints for its behavioral thermoregulation, but not PS because it is concerned with aspects of autonomic thermoregulation.

There is surprisingly little data on the effect of PSD on T_B by which to evaluate the heat loss hypothesis. The results of one human study are consistent with it. Twenty years ago, Foulkes et al. (31) compared the effects of one night of PSD on next-day waking behavior and next-night sleep mentation with the same measures following a night of control awakenings. To disguise the behavioral observations, oral temperatures were taken at 2-h intervals. The authors found it "ironic" that PSD produced no significant experimental-control differences in behavior or sleep mentation, but mean oral temperatures were significantly lower (by 0.23°F) following the PSD night than following the control night. They suggested that the temperature difference might indicate different metabolic functions for PS and NREM sleep. "Ironically" this suggestion was never fully pursued.

Studies of changes in thermal resistance could provide evidence relevant to the heat loss hypothesis. The most direct test would be an evaluation of the effects of PSD on thermal resistance, but we know of no such studies. Three studies in humans evaluated thermal resistance after one to four nights of TSD. In each case, measurement was made near the circadian temperature peak. One study (32) showed lower thermal resistance (lower T_B than controls at the same metabolic rate) at ambient temperatures of 26.7°C and 10°C. A second study (33) showed a trend toward lower thermal resistance (lower T_B than baseline with a higher metabolic rate in three of four 15-min blocks) during exercise at 0°C. Thus, the results of these two studies are consistent, or at least

not at odds with, increased heat loss. The third study (34) showed an initially lower thermal resistance (primarily higher conductivity) than baseline during exercise at 28°C, but resistance increased above baseline (both lower conductivity and less evaporation) as exercise progressed. Therefore, this study suggested both heat retention and dissipation deficits.

There is solid evidence for a lowered thermal setpoint during NREM sleep. Glotzbach and Heller (26) showed in the kangaroo rat that "in comparison to wakefulness, there is a lowered threshold hypothalamic temperature for metabolic heat production and a lowered proportionality constant relating rate of metabolic heat production to hypothalamic temperature." It is possible that the lowered setpoint during NREM sleep persists into subsequent wakefulness. Without the lowering of setpoint during NREM sleep, waking setpoint might be relatively elevated, as in the TSD and HS2D rats.

The literature offers little additional evidence for evaluating the setpoint hypothesis. We know of no studies of the effect of high-amplitude or slow-wave sleep deprivation or of NREM sleep fragmentation on temperature preferences or on T_B. As implied above, TSD studies would be of lesser value, because NREM and PS loss would drive T_B in opposite directions, and predictions would be more ambiguous. The evidence on the effect of TSD on T_B is in fact mixed. Kleitman (35) in 1923 reported that the amplitude of the circadian temperature rhythm declined over (up to 7 days) deprivation, but average temperatures did not change from baseline. In 1987, using a 24-h "constant routine" to minimize effects of extraneous stimuli, Barrett et al. (36) also found a decrease in rhythm amplitude, but this decrease consisted primarily of an increase in T_B during the normal sleep period. Thus, overall T_B increased, presumably because of the loss of the direct ("masking") effect of sleep on T_B. Many of the intervening studies have failed to take adequate account of the circadian variation in T_B, so that reported declines have been with respect to the circadian high in baseline, or not compared to baseline at all. For example, the results of Naitoh et al. (37) have been cited as an example of a T_B decline, but near the end of the 8 days of TSD, all T_B values were near the baseline high values (nighttime low T_Bs were not measured in baseline). Thus, the results might be indicative of a rise in average T_B.

There are several reports on the effects of ambient temperature on sleep, but we will not review them because it is not entirely clear to what extent they are mediated by direct effects on sleep and wake mechanisms—as when animals are made too uncomfortable to sleep—or the response of sleep to thermoregulatory demands. The latter is certainly possible, but thus far our data speak only to issue of the effects of sleep or lack of it on subsequent thermoregulation.

COMPARISON WITH RESULTS OF OTHERS

Extensive comparison of the present results with those of earlier sleep deprivation studies is beyond the scope of this article and of doubtful value in many cases given the great differences in subjects, variables measured, deprivation methods, controls, and especially duration of deprivation. Only a few salient issues will be considered.

Rat studies

Three rat TSD studies (38–40) have reported a weight loss. Four days or less of PSD by the pedestal method has produced variable results on weight change. In four of these

studies (41–44), PSD and PSC rats did not differ in weight change; two studies (45,46) reported a greater weight loss in PSD rats—in one of these (46), the weight loss was combined with greater food intake. Six days of PSD produced a slowing of weight gain in spite of increased food intake (47). Nine days of PSD produced a weight loss in spite of increased food intake (48). A possibly related finding was a short report (49) of highly significant decreases in brain glucose-6-phosphate, highly significant increases in brain ADP and AMP, and a trend toward decreased ATP in PSD rats. This pattern was interpreted as suggesting either an increased utilization of high-energy phosphates or a decreased production of these compounds. The former possibility would be consistent with our observation of high EE. In a similar vein, PSD was reported to reduce brain glycogen content in rats as well as cats (50), but more data would be required to know whether this indicated a decreased supply or increased utilization. As in our rats, Karadzic and Dement (51) reported increased heart rate with PSD.

Two other changes in our TSD and PSD rats appeared relatively early and were readily apparent without special measurement—debilitated appearance and lesions on the tail and plantar surfaces. Therefore, one might have expected some notice of such effects in earlier studies, but they have not been reported. Possibly, these changes were present but dismissed as specific to the deprivation procedure, which in most cases involved either housing the rats on small pedestals in a pool of water or continuous walking on treadmills mounted over water. An embarrassment over method may have also blunted special attention to deaths that occasionally occurred among PSD rats (52,53). Other variables, such as temperature or hormonal changes, either were not measured in earlier sleep deprivation studies of rats, or the deprivation period was too short to expect the same changes that we observed.

Human studies

The other most studied subjects in sleep deprivation studies have been humans. The effects of TSD in humans have been comprehensively reviewed by Wilkinson (54) and more recently by Horne (55,56). From Horne's reviews, it would appear that the major point of correspondence with our TSD data is in several reports of increased hunger in human TSD subjects. Horne (55) cited the recent report of Kant et al. (57) that 72 h of TSD in humans markedly increased urea excretion—a result that parallels the increase in plasma urea nitrogen in our TSD and PSD rats.

Apart from the above, it is probably a fair appraisal to say that neither Horne's nor Wilkinson's reviews reveal any consistent, substantial evidence that TSD humans show the same physiological changes as our TSD rats—either in weight, body metabolism, heart rate, or biochemical measures.

A similar picture emerges from human PSD studies. Increased appetite was noted in Dement's original PSD study (58) and a subsequent report by Sampson (59). Human PSD studies have tended to emphasize psychological, behavioral, and central rather than systemic effects (60), so there is little basis for comparison with the present results. Nevertheless, it is clear that no human PSD or TSD studies have produced the same kind of severe debilitation we have seen in all our deprived rats.

Comparisons of effects of TSD and PSD in our rats and in humans are problematical because deprivation-induced changes probably occur at different rates in different species. The assumption that rates would be similar across species is gratuitous. Other assumptions about species differences in rates are speculative. However, several alternative assumptions derived from other data suggest that humans would not show the

physiological effects of sleep loss as quickly as rats. If one assumes that daily sleep amounts (13.6 h in rats; 8.0 h in humans) reflect sleep need and therefore vulnerability to sleep loss, then one might expect that rats would develop TSD symptoms about 1.7 times faster than humans. By parallel extrapolation from daily PS amounts (2.5 h in rats; 1.9 h in humans), one might expect rats to develop PSD symptoms 1.3 times faster than humans. If it is assumed that the need for PS is better reflected by the shortness of the sleep cycle (sleep time between initiation of successive PS periods: 9.8 min in rats; 90 min in humans), then one might expect rats to develop PSD symptoms 9.2 times faster than humans. If, as the present results indicate, the lethal effects of sleep loss are related to increments in EE, and if the increments are proportional to basal oxygen consumption (0.86 $cm^3/g/h$ in rats; 0.22 in humans), one might expect humans to survive sleep loss about 3.9 times longer than rats. If sleep loss is assessed as a percentage of life span (4.7 years in rats; 100 in humans), then one might expect that rats would succumb to TSD and PSD 21.3 times faster than humans. (All data for the above ratios are from ref. 29.) One hypothesis suggests that some of the major effects of sleep loss are mediated by excessive heat loss. Since rats have a larger ratio of surface area to body mass than humans (940 versus 155 cm^2/kg) (61), they might be six times more vulnerable than humans to such heat loss effects of sleep deprivation. If relative survival times during starvation [16.7 days for rats (3); 61.6 days for humans (62)] are any indication of relative resistance to sleep loss, then we would expect humans to survive sleep loss about 3.7 times longer than rats, or about 77 days of TSD versus 21 days for rats, and 135 days of PSD versus 37 days for rats.

Most human TSD studies have been carried out for 5 days or less; the most notable exceptions are 8.5 days in four young men and 9.2 and 11.0 days respectively in two other young men (reviewed in ref. 56). If humans are assumed to have a similar but less immediate biological vulnerability to TSD as rats, then, by most of the assumptions about temporal course described above, humans have not been subjected to TSD anywhere near long enough to show severe physiological effects. For example, using extrapolations for time course based upon survival during starvation, only one human TSD study of a single subject has progressed beyond the middle of the first quarter of projected survival time, at which point most rats barely show any physiological symptoms. (The estimate based upon survival under starvation is below the median of the ratios cited above.)

Most PSD studies in humans have also been relatively short, in the range of 1–16 nights (see review in ref. 60), and have produced no damaging physiological symptoms. Again using the extrapolations based upon survival during starvation, a 16-night human PSD study would fall just short of the middle of the first quarter of projected survival time. At this point, physiological effects of PSD in our rats were negligible. A few human studies have maintained total or near total and continuous PSD for much longer periods through the use of drugs. Probably the most serious challenge to the generality of our PSD results is the report of Wyatt et al. (63) who induced severe and prolonged PS loss in seven narcoleptic patients by chronic administration of phenelzine, a monoamine oxidase inhibitor (MAOI). Of particular interest were two patients who, on the basis of periodic recordings, had almost complete suppression of PS for more than a year. The patients showed only relatively mild symptoms that were apparently side effects of the drug; e.g., weight gain due to excessive eating. Other studies of prolonged human PSD induced by phenelzine include the following: 14 to 40 days in six anxious-depressed patients (64); 3 weeks in five depressed patients (65); 52 days in one de-

pressed patient and at least 38 days in another (66); "near total" PS suppression for 70 days in a man who suffered painful nocturnal erections (67); 26 days in a man with narcolepsy (68). Additionally, eight depressed patients were subjected to PSD for approximately three to four weeks by the MAOIs clorgyline and pargyline (69). None of these studies reported the kind of severe debilitation we observed in PSD rats. The discrepancy is open to several interpretations:

1. MAOIs might have eliminated the need for PS. Insofar as PS rebounds indicate PS need, this was not the case. In most cases where recordings were continued for several nights after the drug was stopped, large and sustained rebounds were observed. Nevertheless, there could have been some attenuation of the PS need, since the rebounds were much smaller than those we observed in rats (9), and there was no report in the human studies of the sleep onset PS periods we observed.

2. The rebound in humans could have been smaller because the human need for PS is not so urgent as in the rat. As judged by resistance to drug-induced PSD, the need for PS in rats compared to humans may be even greater than indicated by the earlier assumptions. Comparing his attempts to induce PSD in rats with published reports of human studies, G. Vogel (personal communication) estimates that, on a mg/kg basis, 12 times the human dose of imipramine and 25 times the human dose of pargyline were required to completely suppress PS in the rat. Even then, complete suppression could not be maintained in the rat for more than a week.

3. It could be argued that PS has little or no functional value in humans. However, any such argument would have to explain why PS occurs so regularly in humans and why it rebounds following even a few nights of deprivation. One possibility is that the need for PS was obviated in the course of human evolution, but there was no selection pressure to eliminate its occurrence or rebounds. A second possibility is that PS might be critical during human development (28) but not later in life.

4. PS may serve both long-term, well-protected, vegetative functions such as those implicated by our results, and short-term functions that require PS more frequently and that may relate primarily to cerebral activity. There is ample evidence that relatively brief (compared to our studies) PSD affects cerebral activity. In animals, the effects have included decreased thresholds for electrically induced seizures, enhancement of cortical evoked potentials, lowered threshold for and increased response rate of intracranial self-stimulation, and increased sexual and aggressive behavior. In humans, the effects have included enhancement of evoked potentials, intensification of fantasy in fantasy-impoverished subjects, and the relief of endogenous depression (see review in ref. 60). In both animals and humans, effects on learning and memory have also been reported (see review in ref. 70), although some of the findings are controversial. It is possible that only the short-term cerebral functions are urgent for humans, which would account for why they have PS so regularly and show rebounds after PSD but fail to show large vegetative changes. This position has the appeal of encompassing much data. It tempts the further speculation that PS functions to prevent an escalation of both cerebral and systemic excitation. Control of systemic excitation by PS might be urgent only for small animals with high energy costs for thermoregulation and motor activity. [A similar position has been proposed with respect to TS by Horne (55).] However, this view has several weaknesses. First, until we can specify the kinds of excitation and control that are involved, the concept is too vague to be very convincing or heuristically useful. Second, although short-term effects of PSD have been identified, it is not entirely clear that they reflect functional deficit. What is so bad about the elevation of

mood, the enhancement of evoked potentials, or the enrichment of fantasy? Third, in view of the methodological problems of the pedestal method (71,72), short-term effects in animals need confirmation by other methods of PSD. It would therefore be of interest to look for changes in cerebral excitation following short-term PSD by the disk method.

5. All of the above interpretations of human tolerance to prolonged drug-induced PSD are based upon, in one sense or another, a lowered, limited, or nonexistent need for PS. A quite different interpretation suggests that PS may be vital even in adult humans, but that a little may go a long way. In defense of this position, it should first be noted that even in the human MAOI studies, either small amounts of PS were present, or PS equivalents, i.e., cataplexy, occurred (63), or recordings were made only periodically. Our examination of published figures indicates that the longest stretch of consecutively recorded nights with no PS was 21 (64). In contrast, our PSD rats (4) were, on the average, deprived of more than 99% of their baseline PS for 36.6 days. The value of even a little PS for survival was indicated in our first TSD study (15) where, even though mean PS reduction from baseline was 96.4%, the correlation between amount of residual PS and survival time in TSD was $r = 0.94$. In an earlier human study (73), we suggested that "excess" PS may be automatically triggered during NREM sleep as a protection against a state of PS deprivation. The results of our current rat studies suggest that PS may be so essential that it is well protected by excess PS under normal conditions and by high rebound priorities under conditions of deprivation. The implication for the human studies is that the limits of endurance for complete and sustained PSD in humans may not yet have been tested.

It is testimony to our ignorance that interpretations of the human need for PS run the gamut from nonexistent to absolutely vital. The eventual resolution of this ignorance will have to account for the empirical fact that there is a profound difference between rats and humans in the kind and/or rate of response to PSD.

Cat studies

Sleep loss studies in cats are of interest because cats are intermediate between rats and humans on several parameters that may be associated with resistance to sleep loss, including metabolic rate, life span, and ratio of surface area to body mass. TSD times in cat studies have been relatively short. Kiyono et al. (74) subjected cats to TSD for 72 h, but outcome variables were limited to sleep stages, electrophysiological changes, and arousal thresholds. The cats did show the same kind of rebound priority for PS that we found in TSD rats (9). In combination with the rebound data, the fact that in cats and rats PS has the highest arousal thresholds, whereas in humans HS has the highest thresholds raises two alternative possibilities: (a) PS may have rebound priority in cats and rats because it is their "deep" stage, whereas humans may show relative HS rebound priority because it is their "deep" stage. Alternatively, PS arousal thresholds and rebound priorities may be relatively greater in cats and rats because PS is more important for them than for humans and hence must be better protected. Vimont-Vicary et al. (75) did not observe PSD rebound priority in TSD cats. They suggested that the cats of Kiyono et al. may have obtained many undetected "microsleeps" during the deprivation period, but it may also be noted that TSD was maintained for longer periods in the Kiyono study.

Two major PSD studies in cats showed both similarities to and differences from our rat PSD results. Dement et al. (76) observed large increases in eating behavior and heart rate, but there were no reports of mortality. Three cats survived PSD for 25, 32, and 70

days respectively. Vimont-Vicary et al. (75) deprived cats of PS for varying periods up to 26 days. They reported large increases in heart rate during both wakefulness and NREM and a tendency for weight loss while appetite remained about average. The cat deprived for 26 days had previously been subjected to shorter intervals of PSD. After 5 days of apparently unsuccessful recovery from 26 days of PSD, this cat died. The cat's physical condition had deteriorated rapidly after 20 days of PSD. It developed local abscesses of the plantar soles and showed a 19% loss of baseline weight at death. Autopsy revealed hemorrhages in the adrenal glands and a normal macroscopic encephalon. Of all cases of PSD reported in the literature, this one resembled our deprived rats the most. In one short-term study (77), cats subjected to 72 h of PSD did not differ in body weight from control cats.

Dog studies

The majority of extended sleep deprivation studies in species other than rats, cats, and humans were older studies that were done before the discovery of PS and that were therefore limited to TSD. Studies of puppies and adult dogs have pointed to death as a consequence of TSD. Manacéine (78) reported that 10 nursing puppies died within 92 to 143 h of TSD. Like our TSD rats, they showed severe hypothermia and a lowered red cell count. A weight loss was attributed to a loss of appetite. By gentle stimulation or by walking them, Kleitman (79) subjected 12 weaned puppies to TSD for two to seven days to the point of extreme muscular weakness, at which time they were either sacrificed or allowed to sleep. Two of the latter died the following day "in convulsions." Weight loss in some puppies was attributed to a refusal to eat. There was drop in the red cell count but no hypothermia. It is of interest that Kleitman insisted that he was not prepared to say ". . . to what extent the effects of experimental insomnia were due to lack of sleep and to what extent to muscular fatigue." Tarozzi (80) reported that three adult dogs subjected to TSD by walking them when necessary died after 9, 13, and 17 days respectively. Okazaki (81) kept dogs awake by maintaining them in cages with nails sticking out on all sides; they survived 14 to 77 days. Legendre and Pieron (82) subjected dogs to TSD for up to 21 days; but they were sacrificed for histological analyses while they were in good physiological condition. In general, the histological findings in the dog studies were so diverse and sometimes contradictory as to defy simple summary.

Rabbit studies

Early studies of TSD in rabbits focused mainly on histological changes. Crile et al. (83) kept rabbits awake by gentle hand stimulation for 96 to 118 h. They found degenerative changes in cells of central nervous system, liver, and adrenal glands which were largely reversed when the animals were allowed to sleep. Almost in passing, Crile et al. noted 11 cases in which animals died "apparently as a result of insomnia alone." (We cannot figure out from the report the percentage of sleep-deprived rabbits that died.) There were no consistent changes in temperature or weight. Leake et al. (84) reported on symptom formation in 14 rabbits subjected to TSD by housing them in slowly rotating cylindrical cages; 9 rabbits died within 8 to 31 days; 5 were sacrificed on day 31. In describing the results on a larger number of rabbits in the same series of studies, Bast (85) reported that the TSD rabbits showed a gradual weight loss and a gradual increase in pulse rate, but "no marked" variations in temperature until a severe hy-

pothermia developed near the end. Degenerative signs were observed in cells of the medulla, spinal cord, adrenal glands, and thyroid glands.

The net impact of this review, as we assess it, is that there are sufficient indications of mortality, increased food intake, weight loss, and increased heart rate in earlier TSD and PSD studies of animals to make one believe that our results are at least partly generalizable beyond rats and beyond the specific deprivation techniques we have used. The other side of the same coin is that many of the earlier studies, in spite of an absence of controls for stimulation, produced data that pointed to salient physiological features of sleep loss pathology. The greatest challenge to generality is in the failure of TSD and PSD to produce severe physiological symptoms in humans. We believe it can be reasonably argued that TSD has not been enforced long enough in humans to produce the same debilitation we saw in rats. Since TSD probably cannot and certainly should not be enforced "long enough," the issue of generality may have to be resolved by the identification of similar TSD-induced processes in the two species. In the case of PSD, a handful of studies indicate that even very prolonged drug-induced PSD in humans does not produce the same kind of severe debilitation we saw in rats. Here we may have a genuine species difference in response to deprivation. One productive approach might be to determine which species differences are most closely related within species to vulnerability to PSD. Such data could help explain any lack of generality as well as help identify the functional targets of sleep.

ADDENDUM

Subsequent to submission of this paper, we became aware of an earlier paper by Schmidek et al. (86) in which it was speculated that the thermoregulatory insufficiency of PS "might have some beneficial effect in the development and maintenance of optimal functional characteristics of the mechanism of thermoregulation," which "might be correlated to a few ontogenetic and phylogenetic data." These ideas are obviously similar to some of those we have expressed on the thermoregulatory role of sleep.

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