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Research report

Sleep deprivation in the rat by the disk-over-water method

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Abstract

Chronic sleep deprivation may be required to reveal the most serious physiological consequences of sleep loss, but it usually requires strong stimulation which can obscure the interpretation of effects. The disk-over-water method permits chronic sleep deprivation of rats with gentle physical stimulation that can be equally applied to yoked control rats. A series of studies with this method has revealed little or no pathology in the control rats. The deprived rats show a reliable syndrome that includes temperature changes (which vary with the sleep stages that are lost); heat seeking behavior; increased food intake; weight loss; increased metabolic rate; increased plasma norepinephrine; decreased plasma thyroxine; an increased triiodothyronine-thyroxine ratio; and an increase of an enzyme which mediates thermogenesis by brown adipose tissue. The temperature changes are attributable to excessive heat loss and an elevated thermoregulatory setpoint, both of which increase thermoregulatory load, and the other changes are interpretable as responses to this increased load. This pattern indicates that sleep serves a thermoregulatory function in the rat. The sleep deprived rats also show stereotypic ulcerative and hyperkeratotic lesions localized to the tail and plantar surfaces of the paws, and they die within a matter of weeks; the mediation of these changes is unresolved.

Key words: Sleep; Sleep deprivation; Sleep function; Sleep rebound; Paradoxical Sleep, Thermoregulation

1. Introduction

Sleep deprivation (SD) is a potentially useful strategy for studying the function of sleep, but the strategy has its limitations [33]. Because short-term SD may stimulate only sleep-promoting mechanisms, chronic SD may be required to elicit function-revealing deficits. However, the enforcement of chronic SD requires repeated, intrusive stimulation which can blur the interpretation of effects. Do they result from sleep loss or from the strong stimulation used to enforce SD? To simplify communication, we speak of the 'effects' of SD, but strictly speaking, SD studies are correlational. We apply stimuli to enforce SD and report the relationship between the ensuing sleep loss and changes in performance or physiology. However, the changes and the sleep loss could be independent responses to the stimulation. The interpretation that the changes result from sleep loss hinges on minimizing the contribution of the deprivation-enforcing stimulation, which can be especially difficult when strong stimulation is used to enforce chronic SD. To deal with this problem, we designed a procedure [7,35] which uses minimal stimulation to enforce chronic SD in the rat and subjects control rats to the same stimulation without severely limiting their sleep.

2. Experimental paradigm

An experimental (SD) rat and a control rat are simultaneously housed each on one side of a divided 46-cm horizontal disk suspended over a shallow tray of 2–3 cm deep water. EEG, EMG and theta activity are continuously monitored to detect sleep states. When the SD rat starts to sleep or enters a 'forbidden' stage, the disk is automatically rotated at the low speed of 3.33 rpm, awakening the rat and forcing it to walk opposite to disk rotation to avoid being carried into the water. The yoked control rat receives the same mild physical stimulation because it is on the same disk. However, while sleep or targeted sleep stages are severely reduced in the SD rat, the control rat can sleep ad lib whenever the experimental rat is spontaneously awake and the disk is still. Typically, control rats show modest sleep reduction, which probably accounts for the fact that they often show physiological effects in the same direction as those of experimental rats, but to a much smaller degree.

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In one typical study [12] of rats targeted for total SD (TSD), total sleep was reduced by 91% vs. 28% in yoked control (TSC) rats; disk rotation occupied 20% of total time. In one study [22] of rats targeted for selective deprivation of paradoxical sleep (PSD), PS was reduced by 99% in PSD rats vs. 3% in yoked control (PSC) rats; disk rotation occupied 5% of total time. In a second PSD study [24], PS was reduced by 86% in PSD rats and by 13% in PSC rats; disk rotation was at 10%. Rats targeted for selective deprivation of high EEG amplitude NREM sleep (HS2) are referred to as HS2D rats and their yoked controls as HS2C rats. In our only HS2D study [19], HS2 was reduced by 96% in experimental rats and 43% in controls; disk rotation was 16% of total time. We use the term 'targeted' in describing these groups, because TSD was never completely effective, and selective deprivation was never completely selective.

Food and water were available ad lib; cage temperature was maintained at 28–29 °C. To avoid confounds between loss of sleep and disturbance of circadian rhythm, most experiments were on rats adapted to and studied under constant light, which flattens rhythms [10], but similar results were obtained on a 12 h/12 h light/dark schedule [42]. Results from our laboratory on over 170 deprived-control pairs of rats will be summarized.

3. Mortality

Unless deprivation was interrupted, all TSD, PSD and HS2D rats died or were killed when death appeared imminent. Thus, no rat survived unrelenting deprivation of sleep, PS or high voltage NREM sleep; only two of the control rats in all these studies died (from undetermined causes). Mean survival of TSD rats ranged from approximately 2–3 weeks [12,27,32,35,42]. Mean survival was 5 weeks in one PSD study [22]; the deaths of these PSD rats was somewhat remarkable inasmuch as they had more total sleep and much less disk rotation than some TSC rats – another indication that death did not result from the experimental procedures per se. In a second PSD study [24] with less severe PS loss, survival was somewhat longer but not precisely determined because some rats were killed in advance of definite premorbid signs. Mean survival of HS2D rats was 45 days [19]. Although selective deprivation of medium voltage NREM sleep is not possible with known procedures, the shortened survival of TSD rats compared to the selectively deprived rats suggests a vital function for that stage as well.

Because proximal causes of death may result from a cascade of pathologies, they hold less promise for identifying functional targets of sleep than earlier appearing, progressive pathologies. Nevertheless, proximal causes could offer clues about functionally important antecedent pathologies. No severe, uniformly occurring histological abnormalities which might have caused death have been detected in the brains or major systemic organs of SD rats [12,18,22].

Hypothermia had been suspected as a proximal cause of death [3,31,34] because all SD rats showed an eventual decline in intraperitoneal temperature (T_{ip}); a decline to more than 1 °C below baseline in otherwise untreated SD rats has been a reliable indicator of impending death within a day or two. However, TSD rats kept warm by exogenous heating died nevertheless, whereas cold-stressed control rats survived much lower body temperatures than TSD rats [38]. Attenuation of the T_{ip} decline in hyperthyroid TSD rats [5] did not lengthen their survival and acceleration of the decline in hypothyroid TSD rats [31] or TSD rats with lesions of the preoptic anterior hypothalamus [16] did not shorten their survival. Thus, the T_{ip} decline was not necessarily the cause of death in otherwise untreated SD rats.

A second possible cause of death is breakdown of body tissues due to catabolism, secondary to the high metabolic rate in TSD rats [3,14,31]. Evidence supporting a role of catabolism includes significant preterminal declines in jejunal mitosis (cell division) [23] and serum albumin [12] in TSD rats; a shortened survival time in hyperthyroid TSD rats, which had a high metabolic rate and rapid weight loss [5]; and a lengthened survival time in rats on a high calorie diet, which had little or no weight loss [14]. Evidence against catabolism as a mediator of preterminal effects includes a lack of preterminal serum albumin decline in PSD rats [22] and the deaths of all rats in two TSD groups protected against catabolic effects, hypothyroid rats and high-calorie diet rats. Thus tissue breakdown secondary to catabolism was not a necessary cause of death.

A third major candidate for proximal cause of death is organ failure secondary to systemic infection, as suggested by bacteremia, which Everson has observed in five of six TSD rats obviously near death [11]. We subsequently confirmed bacteremia in two additional preterminal TSD rats [20]. Very recently, we treated six TSD rats with antibiotic cocktails; five progressed to an apparently terminal condition nevertheless and were then killed (after 10–16 days of TSD). Neither heart blood samples, livers, kidneys, nor mesenteric lymph nodes showed aerobic bacterial or fungal infection. The sixth rat died after 19 days; blood could not be drawn, but the other tissues were harvested shortly thereafter and were also free of aerobic bacteria and fungi. These results indicate that microbial invasion is not a necessary cause of death in TSD rats.

In summary, lowered T_{ip}, catabolism and bacterial invasion are all correlated with terminal processes in SD

rats and may contribute to their deaths. None of the three, however, has been established as a necessary cause of these deaths. In individual cases, death might result from one or more of these pathologies or other, yet unidentified pathologies.

4. Food intake, weight and energy expenditure

TSD, PSD and HS2D rats showed large increases in food intake combined with weight loss. This pattern did not result from impaired intermediary metabolism. TSD [12] rats and PSD [22] rats showed accelerated use of fats and proteins, accelerated (TSD) or normal (PSD) glucose uptake and no evidence of glucosuria. Neither was there calorimetric evidence of increased energy loss in waste products or of weight loss from dehydration [4]. These results indicated that the food and weight changes resulted from increased metabolic rate, which was confirmed by indirect calorimetry (doubly labelled water method) [4]. Accordingly, we have used the caloric values of food intake and weight change to routinely derive energy expenditure (EE) as a less expensive, less intrusive, indicator of metabolic rate. All SD rats showed increases in EE which tended to be progressive across deprivation and eventually became quite large. Four TSD studies [12,27,32,42] showed mean EE increases which reached 210%–270% of baseline near the end of deprivation. The stage-selective deprivation studies produced EE increases within the same range. Control rats generally showed much smaller, significantly lower increases.

Did the EE increases in SD rats result from overly active calorigenic processes? Blood levels of calorigenic hormones were tracked in TSD-TSC and PSD-PSC rat pairs [4]. Thyroxine was decreased in SD rats, both with respect to baseline and relative to controls. ACTH changes were not significantly different in SD and control rats. Corticosterone was similar in PSD and PSC rats, but a small increase in TSD rats was significant vs. a decline in TSC rats. This difference did not appear until late in deprivation, well after large group differences in EE were apparent. Epinephrine increased more in TSD rats than in TSC rats, but again the difference did not appear until late in deprivation; PSD and PSC rats did not differ significantly. The most substantial deprived-control differences were in norepinephrine (NE). In both TSD and PSD rats, the NE increase started early, increased progressively and was eventually much greater than in controls. Thus, the best candidate for an overly active calorigenic mechanism was NE-mediated sympathetic activation.

To evaluate the role of NE-mediated sympathetic activation on increased EE and other symptoms of sleep deprivation, the peripheral sympathetic blocking agent guanethidine was administered to TSD rats [30]. As expected, guanethidine attenuated the NE increase previously seen in TSD rats, but the increase in EE was not attenuated. (Neither was there an effect on TSD-induced changes in appearance, skin condition or temperature, which will be reviewed later.) On the other hand, the guanethidine-treated TSD rats, showed significantly greater increases in circulating epinephrine than untreated TSD rats. The results suggested the substitution of one calorigenic mediator (epinephrine) for another (NE) in response to an abnormally elevated *need* for energy.

Several studies were directed to the issue of what might have elevated the energy need in SD rats [4]. Locomotor activity, was not significantly different in SD rats and controls. Immersions in pan water surrounding the disk could not have accounted for the magnitude of the EE increase seen in SD rats. Until they were near death, the rats usually *avoided* the water by walking opposite to disk rotation. Based on videotape samples, mean *total min per day* during successive quarters of TSD survival time were: for partial immersions (two or three paws in water) 4.4, 23.9, 27.3, 41.2; for full immersions (four paws and sometimes torso) 0, 0.4, 1.1, 1.1. The corresponding data for PSD rats were 10.7, 33.5, 22.3 and 15.5 min for partial immersions and 0.7, 1.2, 0.8 and 4.7 min for full immersions. EE increases in control rats immersed in pan water as frequently and for even longer durations than SD rats were much smaller than those of SD rats; water immersion could have accounted for only a small fraction of the EE increase in the SD rats. It is also unlikely that increased EE was required to sustain the metabolic expense of increased wakefulness. Although the increase of wakefulness over baseline levels remained fairly stable over the course of SD, EE increased progressively. In the PSD experiment, heart rate could be recorded during both wakefulness and NREM sleep. During PSD, both NREM and waking heart rates increased; during the last three quarters of deprivation, NREM heart rate was above the *waking* baseline level. Most likely, the EE increase in SD rats consists primarily of an increase in resting metabolic rate.

Extrapolating from evidence of bacteremia in preterminal TSD rats [11], Everson speculated that SD-induced increases in EE might be mediated by cachexic cytokine responses to impaired host defense. We recently found increased EE in TSD rats with no impairment of host defense as measured by bacterial invasion of blood, liver, kidney or mesenteric lymph nodes or by bacterial adhesion to the cecum [20]. This result does not rule out a possible, as yet unexplored role of cachexic cytokines in the EE increases, but, given that these cytokines also produce hypophagia, such a role is unlikely.

5. Thermoregulation

There is evidence that energy need in SD rats increases in response to SD-induced thermoregulatory changes which increase metabolic load. In two PSD studies [22,24], T_{ip} declined progressively while EE increased progressively. Heat must have escaped the body at a progressively higher rate. The heat loss was 'excessive' inasmuch as the rats chose progressively higher ambient temperatures in a thermocline, were piloerected and showed signs of peripheral vasoconstriction (blanching of the ears, tips of the paws and mucous membranes of the mouth), i.e., T_{ip} declined below temperature setpoint (T_{set}) [24].

Temperature changes in TSD rats could not be explained by excessive heat loss alone. TSD rats showed an initial increase in T_{ip} followed by a later fall to below baseline [4,27,32,37,42]. Several lines of evidence indicate that the T_{ip} increase was stimulated by an increase in T_{set}. (a) TSD rats showed a strong preference for high ambient temperatures in a thermal gradient [32] and increased operant responding for ambient heat [37] even early in deprivation when T_{ip} was above baseline. (b) As T_{ip} declined toward baseline, EE continued to rise, an apparent metabolic compensation for the retreat from the elevated temperature. (c) Hypothalamic temperature (T_{hy}), generally considered to be more tightly regulated than T_{ip}, was maintained above baseline even when T_{ip} dropped [27,37]. TSD rats also suffered excessive heat loss as indicated by the decline of T_{ip} and eventually of T_{hy}, to below preferred and baseline temperatures in spite of greatly elevated EE. Although increased T_{set} could be most conclusively demonstrated for the early portions of deprivation and excessive heat loss for the later portions, most likely both thermoregulatory changes obtained throughout most of deprivation [27].

Quantitative estimations of T_{set} based upon values of T_{ip} and preferred ambient temperatures suggested that PSD rats did not have an increase in T_{set} [24]. Their increases in preferred ambient temperature were apparently targeted to maintain baseline T_{ip} rather than an elevated T_{set}. In TSD rats, T_{set} is apparently higher for T_{hy} than for T_{ip} [27,37]. We have not yet measured T_{hy} in PSD rats, so we do not know whether its T_{set} may be elevated. Based on T_{ip} data, however, it is very unlikely that T_{set} for T_{hy} in PSD rats would be as high as in TSD rats. Therefore, it seems likely that the increase of T_{set} in TSD rats is attributable *primarily* to a loss of NREM sleep. Consistent with this interpretation, in HS2D rats, which were targeted for selective deprivation of high voltage NREM sleep, T_{ip} remained above baseline until very shortly before actual or anticipated death [19].

The effect of SD on temperature regulation is revealed not so much by temperature levels, which usually remained within 1 °C of baseline, as by the magnitude of the thermoregulatory adjustments. By the fourth quarter of deprivation, TSD rats initially selected mean air temperatures of 50 °C (hot to the touch) in a thermal gradient [32], self-regulated mean cage temperature at 37 °C compared to 26 °C during baseline [37] and more than doubled baseline EE. That the increase in EE was at least in part a thermoregulatory adjustment to the raised T_{set} and excessive heat loss is indicated by a study of TSD rats in which the rise in EE was blunted by making them hypothyroid; T_{ip} did not show the characteristic early rise and fell much more quickly than in euthyroid TSD rats [31]. The use of water-immersion controls in this study demonstrated that the T_{ip} decline did not result from water exposure. In both TSD and PSD rats, the positive correlation between EE and preferred ambient temperature is consistent with an elevation of thermogenesis and behavioral warming in response to a heat retention deficit [24]. If the primary pathology had been an increase in EE, then one would have expected behavioral cooling. The hormonal profile — including increases in NE [4], the ratio of triiodothyronine (T3) to thyroxine (T4) [4], and brown adipose tissue type II 5′-deiodinase [1] activity — is also consistent with compensatory thermogenesis.

Relatively little progress has been made on how the above SD-induced thermoregulatory changes are mediated. Because TSD rats decreased T_{ip} in response to the α-adrenergic antagonist phentolamine, it appeared that noradrenergically mediated vasoconstrictors had been intact prior to phentolamine injection [28]. To determine whether the increase in T_{set} was mediated by prostaglandins, TSD rats were given aspirin, which blocks prostaglandin synthesis [8]. The TSD-induced increase in T_{ip} was reduced significantly, but only by 26%. Opioids may also increase T_{set}, but naltrexone, a receptor antagonist, had little effect on the TSD-induced T_{ip} increase [43]. Lesions of the preoptic anterior hypothalamus (POAH) which were sufficient to reduce homeostatic thermoregulatory responses to variations in ambient temperature produced no substantial changes in T_{ip} or EE at the thermoneutral ambient temperatures which prevailed in TSD studies, making it unlikely that POAH impairment alone could have accounted for TSD effects [16]. POAH lesions exacerbated TSD-induced T_{ip} declines. This result suggests that the POAH normally regulates temperatures in a narrow range against the excessive heat loss produced by TSD-induced impairments of other, yet unidentified thermoregulatory mechanisms.

6. Skin, fur and appearance changes

TSD [12], PSD [22] and HS2D [19] rats showed a progressively debilitated appearance manifest in dishev-

eled, clumped, yellowing fur, even though grooming was not reduced. They also developed severe ulcerative and hyperkeratotic lesions localized to the planter surfaces of the paws and to the tail [23]. Yoked control rats, which endured the same mechanical pressures and water immersion, had significantly better appearance and little skin pathology. It is unlikely that infection was the primary cause of the lesions or that the lesions were an important portal for microbial invasion, because bacterial clumps in the fibrinoid crust were confined to the surface layer of the skin, as might be expected from exposure of the wound, and there was no necrotizing vasculitis.

There was mixed evidence on whether these lesions were associated with catabolic processes. Appearance changes and lesions were mild or absent in hypothyroid TSD rats [31] and delayed in high-calorie TSD rats [14], groups which were presumably protected against catabolism. However, hyperthyroid TSC rats, which had high EE levels and rapid weight loss also did not develop severe lesions or a debilitated appearance [5], nor did chronically food deprived rats [12]. The mediation of the lesions and appearance changes remains unexplained. Because T_{ip} declined even faster in hypothyroid TSD rats than in euthyroid TSD rats, the skin changes are not a prerequisite for the excessive heat loss.

7. Host defense

Benca et al. [2] tested splenic lymphocytes collected from preterminal TSD and PSD rats for their in vitro response to antigen challenges and mitogens. There were no significant reductions in either antibody production or lymphocyte proliferation responses to a variety of mitogens. TSD and PSD rats injected in vivo with antigens also showed no significant suppression of antibody responses. These results contrast with Everson's demonstration of bacteremia in preterminal TSD rats [11], which is definitive evidence of impaired host defense. The Everson and Benca results would not be incompatible if it turned out that the impairment of host defense resulted from a preterminal breakdown of innate tissue barriers to microbial translocation, as implied by the decreased preterminal jejunal mitosis [23], rather than from impairment of lymphocyte function. We found only a statistically nonsignificant tendency for greater bacterial adhesion to the cecum (a measure of gut barrier integrity) in rats killed after 4 days of TSD than in their yoked controls [20]. In both groups, there were no aerobic bacteria in livers and kidneys and none or background levels in the mesenteric lymph nodes, i.e., there was no indication of bacterial invasion in the major organs of filtration.

Recently we found significantly less tumor growth following subdermal injection of cancer cells in rats subsequently subjected to 10 days of TSD than in their yoked controls (Bergmann, B.M. et al., unpublished results). It is becoming increasingly clear that SD studies are not going to produce uniform enhancement or impairment of host defense; some host defense mechanisms may be enhanced whereas others are impaired or remain unchanged.

8. Brain changes

There were no systematic histological differences at the light microscope level between the brains of TSD and TSC rats [18]. Monoamines have been implicated in sleep regulation, but effects of SD on brain monoamines have been varied and sometimes contradictory. We found no significant differences which could be unambiguously attributed to sleep loss between rats subjected to TSD for 11–20 days and their yoked controls in regional brain levels of serotonin, norepinephrine, dopamine or their major metabolites [9]. Because one well-conceived theory proposed that PS served to upregulate noradrenergic receptors [39], we examined the effect of 10 days of TSD (which includes PSD as well as PS-like activity in NREM sleep) on regional brain levels of adrenoceptors [40]. We found no TSD-TSC differences which could unambiguously be attributed to sleep loss. Because acetylcholine plays an important role in the generation of PS and because chronic SD produces huge rebounds in PS (see below), we evaluated whether 10 days of TSD upregulated brain cholinergic receptors [41]; there was little evidence to that effect. The immediate early gene Egr-1 is a sensitive, regionally specific marker of post-synaptic excitation, but we found no large scale global brain changes in its expression as a result of 10 days of TSD – only suggestions of a few regionally specific changes [25]. It is difficult to believe that 10 days or more of SD, which is sufficient to produce major changes in thermoregulation, metabolism, and circulating hormones, does not also produce definitive changes in some parameter of brain function that is relevant to sleep function or mechanisms. Most likely, we have not targeted the critical parameters or examined the sleep-related brain regions in sufficient detail.

9. Recovery

If SD was halted before Tip declined too far and if the rats were not so debilitated that they could not sustain extended sleep bouts, they recovered from most of the SD-induced effects. Recovery was first studied for at least 15 days in three 14–21 day TSD rats maintained in constant light [13] and then for 11 days in three 19–21 day

TSD rats maintained on a 12 h/12 h light/dark schedule [42]. In both studies, EE returned to near or, in most cases, slightly below baseline on the first recovery day. Tail and paw lesions showed signs of scab formation on the second recovery day and were almost completely healed by the end of the recovery period. Appearance gradually returned to normal. Recovery of blood hormone levels was evaluated only in the first study [13]. As in our earlier hormone study [4], thyroxine decreased significantly while epinephrine and NE increased significantly during the course of deprivation; all returned to near baseline levels during the recovery period. Excepting a small dip early in recovery, ACTH did not change substantially across baseline, deprivation and recovery, which was not surprising in view of the absence of significant TSD-TSC differences in the earlier study [4]. Corticosterone declined non-significantly through deprivation and early recovery, which was also not surprising in view of its erratic course during deprivation in the earlier study [4]. Mean waking T_{ip} returned to baseline on the first recovery day of the first study and by the fourth recovery day in the second. Recently, longer-lasting T_{ip} effects were discerned [17]. Rats which had been subjected to TSD for 14–21 days were recorded for 8 recovery days. Waking T_{ip} showed a substantial but incomplete increase towards baseline levels on the first recovery day, while the sleep T_{ip} rose to slightly above baseline levels. The net result was elimination of the typical wake-sleep difference in T_{ip}. In fact, some of the rats showed increases in T_{ip} in the passage from wakefulness to sleep. As recovery continued, there was a tendency towards restoration of typical wake-sleep differences, but the restoration was still incomplete at the end of the 8-day recovery period. The duration of this effect emphasizes the impact of SD on thermoregulation. In 5-day TSD rats, recovery began while T_{ip} was still elevated. Waking T_{ip} declined at the start of recovery, but sleep T_{ip} was above baseline levels and again the sleep-wake difference was eliminated – in this case only for the first recovery day. The combined results suggest that during recovery from TSD the normal drop in T_{set} from wake to sleep is reduced or reversed and that this change is more prolonged following more prolonged TSD.

TSD rats showed large rebounds of PS and no substantial rebounds of NREM or HS2. (The single HS2D rat studied during recovery [13] did show an HS2 rebound.) On the first day of recovery from TSD, PS was 9.9 times greater than baseline in the first recovery study [13] and 4.4 times greater in the second [42]. PS subsequently decreased but remained greater than baseline throughout the recorded recovery. For the recovery period as a whole, PS averaged twice baseline in both studies. There was a modest total sleep rebound in most rats, but it consisted primarily of the augmented PS. The priority for PS rebound and the absence of an early HS2 rebound contrasts with the early HS2 rebounds, sometimes concomitant with PS rebounds, in shorter duration TSD studies (see references in [13] and [15]). Insofar as rebound priorities may indicate sleep need, the high priority for PS rebound in chronic TSD rats suggests that the need for PS may exceed the need for NREM when PS loss has been severe or prolonged or when survival is at stake. The paucity of NREM rebound might be explained by the suggestion of Feinberg and Campbell [15] that PS represents a state of disinhibited neuronal activity that 'depletes' the system responsible for EEG delta activity. In any event, the large PS rebounds of TSD rats, which had suffered large, sustained reductions in NREM sleep, challenges the view that PS is a response to processes which occur in NREM sleep.

10. Stress vs. specific deprivation effects

The term 'stress' is often used indiscriminately to describe almost any insult to an organism or the responses to an insult. Unless 'stress responses' are defined by their non-specific occurrence during a wide variety of insults, the term can obscure functional information, e.g., the observation of dehydration in response to water deprivation implies more than that a stressor was applied. Therefore, it is important to resolve whether the changes in SD rats are specific to sleep loss or whether they are stress responses which occur in a wide variety of noxious situations.

One way to approach the issue is to examine whether well-established stress responses are seen in SD rats. The best established stress responses are those which derive from activation of the hypothalamic-pituitary-adrenal (HPA) axis. As reviewed previously [34], indicators of this activation such as increased ACTH and corticosterone, adrenal hypertrophy and stomach ulcers were either absent, minimal or poorly correlated with the development of changes in SD rats. For example, of four SD studies where corticosterone was measured [4,35,13], only one TSD study [4] showed a rise with a significant TSD-TSC difference and that resulted in part from a decrease in the TSC rats. In that study, corticosterone levels were poorly correlated with TSD symptoms; they were only 2% above baseline during the second quarter of deprivation, when changes in EE [12], appearance [12], T_{ip} [4] and behavioral warming [30,37] are all clearly evident. Thus, HPA activation is not necessary to produce SD effects.

If one wished to infer stress in SD rats on the basis of stimulus conditions independent of HPA activation, then it would be important to ask whether the SD effects routinely occur in response to other stressful stimulus condi-

tions. SD effects constitute a reliable syndrome. With the exception of the special case of hypothyroid TSD rats, whenever the relevant parameters have been measured and SD was sufficiently long, *every* SD rat we have ever studied has shown *every one* of the following syndrome components: increased food intake; weight loss; increased EE; a progressively debilitated appearance; stereotypic ulcerative and hyperkeratotic skin lesions localized to the tail and plantar surfaces of the paws; increased plasma norepinephrine; decreased plasma thyroxine; eventual death (unless SD was halted or the rat was killed when death appeared imminent). This syndrome is not a routine response to non-specific stress. In fact, it would be reasonable to ask whether any rat, stressed or not, showed this syndrome without having been subjected to chronic SD; we do not know of any. Since SD rats suffer excessive heat loss, they show some of the changes seen in chronically cold stressed rats, e.g., the hormonal profile, the increase in metabolism, the preference for warmer ambient temperatures. However, the SD rats show these changes at thermoneutral ambient temperatures, and the cold stressed rats do not show the stereotypic skin lesions. Neither do they die until they become much more severely hypothermic. Two TSC rats maintained at 16 °C ambient temperature showed T_{ip}'s more than 0.5 °C below baseline during most of the experimental period. Nevertheless, they survived for 34 days, longer than any TSD rat, before they were killed [38].

Temperature changes are seen in stressed rats. However, TSD rats showed temperature increases followed by temperature decreases, PSD rats showed only decreases and HS2D rats showed mostly temperature increases. All these temperature changes cannot be non-specific stress responses, since they are specific to the kind of sleep loss.

In the most prolonged stress study of rats with frequent presentation of noxious stimuli we have been able to locate, Paré [29] administered 5 s of shock at intervals averaging 5 min for 22 h a day for 23 days. The group with the greatest stress response, as indicated by elevated adrenal weights and adrenal ascorbic acid, was one in which shocks were paired with warning tones. This group suffered a weight loss of about 8.5% compared to a weight loss of 17.3% in TSD rats [12]. More to the point, weight loss in Paré's rats accompanied a 45% *decrease* in food intake, whereas the TSD rats suffered greater weight loss with a 75% *increase* in food intake. Paré described no skin lesions such as we described; these lesions are obvious; they could not possibly be missed by an investigator looking at physiological effects of stress. All of Paré's rats survived until they were killed for assays.

There are risks in both underestimating and overestimating the role of stress. In our opinion, the greater risk by far in evaluating the SD syndrome is in overestimating the role of non-specific stress, thereby erroneously discounting important physiological effects of SD.

11. Generality of results

The generality of the results has been reviewed previously [34]; only a few salient points will be repeated here. Comparison of the present results with other SD studies is problematic because of differences in method, species, parameters examined and especially length of deprivation. Nevertheless, there is scattered evidence of similar results in other SD studies of animals. The greatest challenge to the generality of the present results is the apparent absence of similar, severe physiological symptoms in humans whose PS (REM sleep) had been chronically restricted by antidepressant medication. Many of the SD symptoms we observed related to changes in thermoregulation. Perhaps, following a line of reasoning suggested by Horne [21], the thermoregulatory function of sleep may be important only for small animals with a high thermoregulatory load.

Thermoregulation may have to be periodically reduced or suspended during sleep to maintain long term effectiveness. The high negative correlations between sleep quotas and size and age may derive from the high thermoregulatory loads in small animals and the young. Reptiles may need NREM sleep to establish setpoints for behavioral thermoregulation, but they may not need PS because they can tolerate substantial heat loss.

12. Summary

Rats chronically deprived of sleep by a procedure which subjects yoked control rats to the same benign physical stimulation showed physiological changes which were minimal or absent in the control rats. The deprived rats showed evidence of excessive heat loss and increased T_{set}, both of which increase thermoregulatory load. The excessive heat loss was most closely associated with the loss of paradoxical sleep, whereas the increase in T_{set} was most closely associated with the loss of NREM sleep. The deprived rats also showed large increases in metabolic rate and hormonal changes which are interpretable as responses to the increased thermoregulatory load. These results indicate that sleep has thermoregulatory functions, a view shared by others (e.g., [26,36]). Because we observed temperature and thermoregulatory changes during wakefulness, it would appear that the thermoregulatory function of sleep extends beyond the effects of lowered sleep temperatures to long-term temperature regulation in wakefulness.

All rats subjected to unrelenting total sleep deprivation

died, usually after 2–3 weeks. Rats subjected to selective sleep stage deprivation survived longer, but also died. Evidently sleep and its substages serve vital functions. Lowered body temperatures, high metabolic rates and bacteremia were all correlates of the terminal condition, but none of these was a necessary cause of death in these animals.

The chronically sleep deprived rats also showed ulcerative and hyperkeratotic lesions localized to the tail and the plantar surfaces of the paws. The mediation of these lesions is unclear.

If sleep deprivation was halted before the rats became moribund, almost all deprivation effects were reversed, but there was a persistent reduction of typical sleep-wake temperature differences during recovery from extended deprivation. Recovery featured very large and persistent rebounds of PS, suggesting a functional priority for this stage following extended sleep deprivation or its serious physiological effects.

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