# Sleep Deprivation in the Rat: An Update of the 1989 Paper

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## CONFIRMATION OF EARLIER RESULTS

THE REPRINTED REPORT[1] LISTED SEVERAL MAJOR SLEEP DEPRIVATION EFFECTS (SDES) WHICH WERE APPARENT IN ALL RATS subjected to prolonged total sleep deprivation (TSD) or paradoxical sleep deprivation (PSD) by the disk-over-water (DOW) method. The following original effects were confirmed in subsequent studies:

1. Mortality: Unless deprivation was halted, all TSD rats died or showed signs of impending death—usually in about two to three weeks.[2-10] The deaths (after about four to six weeks) of PSD rats were also confirmed.[11,12]

2. TSD and PSD rats lost weight in spite of increased food intake. The large rise in energy expenditure (EE), calculated from the caloric values of food intake and weight loss) was confirmed.[2,4-9,13-17]

3. The development of scrawny, debilitated appearance was confirmed.[2,4-7,11]

4. The severe ulcerative and hyperkeratotic skin lesions localized to the paws and tails of TSD and PSD rats were confirmed.[2,4-7,9,11]

5. As in the original studies, TSD rats showed an initial rise and subsequent decline in waking intraperitoneal temperature ($T_{ip}$).[2,4,5,10,11,14,16] As before, PSD rats showed only the $T_{ip}$ decline.[11,12]

6. As in the earlier report, recovery from extended TSD featured large rebounds of PS.[6,16] Recent studies showed a predominance of PS rebound after only two[18] or four[19] days of TSD.

Altogether, the confirmatory studies showed that TSD and PSD produce a reliably elicited syndrome of major biological effects.

## POSSIBLE CONFOUNDS

The introduction to this series of papers[20] emphasized that sleep deprivation studies are essentially correlational. We administer stimuli that produce the responses of sleep reduction and the responses of physiological or behavioral change. These are correlated responses. We cannot confidently interpret the physiological and behavioral changes as effects of the sleep loss until we can discount the plausibility of other putative mediators of the physiological and behavioral change that are produced by the experimental situation. Two alternative mediators have been suggested: loss of circadian rhythm and stress.

### Circadian Rhythm

Because sleep deprived rats might have weaker circadian rhythms than control rats, all our experiments had been run in constant light to flatten the circadian rhythms of both sleep deprived and control rats. However, this procedure caused at least one journal reviewer to question whether the effects we reported might have resulted from an interaction of rhythm and sleep loss (i.e., the SDEs might occur only when circadian rhythms were flat). Because we knew of no effects of circadian rhythm loss that resembled the SDEs, and because there was no appealing rationale for the suggested interaction, we doubted its plausibility. Nevertheless, to dispose of such questions, we repeated the basic TSD disk-over-water experiment under 12:12 light-dark conditions.[6] All the major SDEs that we had previously reported were still present, including: increased food intake; decreased weight; increased EE; debilitated appearance; lesions on the paws and tail; an initial increase followed by a large decrease in $T_{ip}$; impending death; and recovery which featured large, sustained rebounds of PS and reversal of all TSD-induced changes.

### Stress

Because it is equally administered to experimental and control rats, the physical stimulation of the DOW experiment is controlled for stressors. However, the SDEs might be stress responses rather than specific functional consequences of sleep loss. If SDEs were much the same as nonspecific responses to a large variety of noxious situations, they would reveal nothing specific about sleep function. Empirically, the issue boils down to whether the SDEs are similar to generally accepted "stress" effects or whether they are relatively unique. Evidence on this issue was presented in three different reports; the review paper republished here;[1] a 1985 summary of our work to that point;[21] a recent response to a "stress" interpretation of our results.[22] Briefly, the evidence is overwhelmingly against the stress interpretation. Deprived rats showed either minimal or none of the following "stress" indicators listed by Selye and others: stomach ulcers; adrenal hypertrophy; increases in ACTH and corticosterone; decreased food intake (deprived rats increased food intake); shrinking of the spleen; reduction in capillary blood flow; initial decreases in metabolic rate; initial hypothermia and

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later fever (TSD rats showed the opposite (i.e., initial increases and later decreases); expression of stress-response genes. On the other hand, sleep deprived rats show a unique syndrome of progressive energy increase, distinctive skin lesions, thermoregulatory changes, and eventual death that we have never seen described in any stressed rats.

All rats permitted to recover from disk-over-water sleep deprivation have shown large rebounds of PS. Some stress studies have also reported large increases or rebounds of PS, but other stress studies have reported decreases or no change—depending on the stressor used, the stress indicator used, and the duration of stress. Overall, there is little to indicate that the SDEs resulted from stress or damping of circadian rhythms.

## NEW FINDINGS

As noted above, TSD rats had shown an initial increase and subsequent decline in waking $T_{ip}$. Because EE had increased, the late decline appeared to result from increased heat loss. Since the 1989 report, new studies confirmed earlier suggestions that the initial waking $T_{ip}$ increase in TSD rats resulted from an increase in temperature setpoint ($T_{set}$). Hypothalamic temperature ($T_{hy}$), which is considered more tightly regulated than $T_{ip}$, remained above baseline longer than $T_{ip}$.[4,10] TSD rats selected progressively higher ambient temperatures (up to 49.9°C) in a thermal gradient.[5]

Progressively increased operant responding for heat raised mean ambient temperature to as much as 9°C above baseline.[10] Self-selected ambient temperatures and operant responding for heat were increased even when $T_{ip}$ was above baseline, indicating that they were not simply responses to low body temperatures.

PSD rats did not appear to have a raised $T_{set}$. In contrast to TSD rats, they had not shown an initial elevation in waking $T_{ip}$, nor did they show an initial increase in $T_{hy}$ in later studies.[12] Although PSD rats showed increases in self-selected ambient temperatures, quantitative analysis indicated that these were targeted to compensate for falling $T_{ip}$ rather than an increased $T_{set}$,[11] These results indicated that the increase in $T_{set}$ in TSD rats had resulted from the loss of NREM sleep. In rats targeted for the selective deprivation of high voltage NREM sleep, $T_{ip}$ remained above baseline until just before actual or impending death,[23] suggesting that PS loss was the major factor in the excessive heat loss of TSD rats.

A third thermoegulatory effect of sleep loss was first noted serendipitously in a PSD study;[11] $T_{ip}$ did not decrease as much during the transitions from wake to NREM sleep as it had during baseline. A subsequent PSD study[12] showed that the effect was so strong that not only was the decline of $T_{ip}$ and $T_{hy}$ during NREM sleep reduced as PSD progressed, but, by the last quarter of the deprivation period, the direction reversed and temperatures progressively increased over the sleep period. Another study[16] showed that the effect could be relatively long lasting. $T_{ip}$ and $T_{hy}$ differences between wake and sleep were virtually absent at the beginning of recovery from TSD, and it took several days of recovery before normal temperature declines during sleep gradually returned.

Thus, DOW studies have revealed three thermoregulatory effects of sleep deprivation: an increase in $T_{set}$ which results from a loss of NREM sleep, and excessive heat loss and an attenuation of normal wake-sleep temperature differences that can result from PS loss alone but may also be affected by NREM loss. The overall message of these three effects is that, quite apart from the possible energy savings of temperature reduction during sleep, at least in the rat, sleep is necessary for the normal operation of temperature-regulating mechanisms during both sleep and wakefulness.

## MEDIATION

The DOW method successfully enforced sleep deprivation long enough to elicit the kind of profound changes that would be expected from blocking a vital function. Just what we had been looking for, but not enough by a long shot! As we contemplated our initial findings, we came to appreciate more and more that the effects of sleep deprivation alone will not tell us the function of sleep. (Would we be happy with the conclusion that the function of eating was to prevent an increase in appetite?) We need to know much more. First, from among the several effects of sleep loss, we need to decide which are direct, primary functional deficits of sleep loss which are compensations for the primary deficits, and which are sequella of the primary effects but not in and of themselves primary functional targets of sleep. Once the primary deficits are identified, attention may be directed to the processes (or absence of processes) that produce those effects. And finally, and most important, what is it specifically about sleep that fosters or blocks the critical processes? In the years since the1989 review, we have made some progress toward sorting out relationships among the different effects, very modest progress toward deciphering their mediation, and have not even begun to understand what it is about the physiology of sleep that is necessary to prevent the SDEs. The successes and failures of our own research efforts since the 1989 publication have thoroughly impressed us that although sleep deprivation effects may be used as a clues to critical functional processes, a simple deficit=function equation is sadly naive.

### Energy Expenditure

The approximate doubling of EE in virtually all DOW sleep deprived rats naturally attracted attention to how it was mediated and how it related to the other SDEs. The 1989 studies had shown that the increase in EE above yoked control levels could not readily be explained by impaired intermediary metabolism, a loss of energy in wastes, water exposure, or motor activity. Neither could the increase in EE be easily explained by the metabolic expense of increased wakefulness; time awake remained fairly stable over the course of sleep deprivation while EE increased progressively and dramatically. In PSD rats, heart rate during NREM sleep rose to above baseline waking levels. An increase in resting EE was indicated and confirmed by indirect calorimetry. Was EE elevation driven by very high levels of calorigenic hormones? The profiles of corticosterone, thyroxine, and epinephrine over the course of deprivation did not recommend them as candidates, but circulating norepinephrine started increasing early and continued to increased progressively and substantially over the course of deprivation, suggesting that norepinephrine-mediated sympathetic activation might be an important mediator of the EE increases.[24]

In one of the first studies undertaken following the 1989 studies (and noted briefly in the 1989 paper), we administered the peripheral sympathetic blocking agent guanethidine to TSD rats to determine whether it would block the EE rise and how that might affect the other SDEs.[2] The increase in norepinephrine was attenuated as expected, but the increase in EE was not. Neither were the other SDEs substantially affected. The major difference was a much larger increase in circulating epinephrine than had been seen in the original, untreated TSD rats. It appeared as though the EE increases had been maintained by the substitution of one calorigenic mediator for another, suggesting that the EE increase was not the result of an abnormal, runaway metabolic mechanism, but a response to an abnormally high need for energy.

To identify which needs the EE increase might be serving and which SDEs it might be sustaining, another method was used to blunt the EE increase. TSD rats were made hypothyroid by administering propylthiouracil, which inhibits the formation of thyroid hormones.[3] By this procedure, EE in TSD rats was held to near pre-drug baseline levels. In contrast to the original euthyroid TSD rats, the hypothyroid TSD rats did not show the early increase of $T_{ip}$. Because they selected even higher ambient temperatures in the thermal gradient than euthyroid TSD rats, it would appear that their $T_{set}$ remained elevated, buy EE was insufficient to raise $T_{ip}$ to the elevated $T_{set}$. In a similar vein, the decline of $T_{ip}$ over the course of deprivation started earlier, was steeper, and reached lower levels in the hypothyroid TSD rats than in the the untreated TSD rats. Indications of piloerection and shivering were also much greater. That the need for energy was unsatisfied in the hypothyroid TSD rats was also suggested by their extremely high plasma levels of epinephrine and norepinephrine, which, however, were unsuccessful in elevating EE because these hormones require the permissive action of thyroid hormones to increase calorigenesis. The pattern of results indicates that the high EE in the original untreated TSD rats had functioned to elevate body temperatures toward a raised $T_{set}$ and to generate energy to compensate for excessive heat loss. Additional evidence of compensatory thermogenesis in these rats was direct conversion of stored energy to heat by activation of brown adipose tissue (BAT). This conversion was indicated by increases in BAT type II 5'-deiodinase (M), which catalyzes the conversion of thyroxin (T4) to its active form, tri-iodothyronine (T3), thereby producing the elevated $T_3/T_4$ ratios[24] reported in the 1989 papers (See also[25]).

Although the high EE provided needed heat, there had been indications that its effects were not entirely salutary. In TSD, PSD, and HS2D (deprivation of high amplitude NREM sleep), high rates of increase in EE during the first half of deprivation were highly correlated with shortened survival time. To evaluate the effects of high EE on other SDEs, we increased it by the administration of thyroxine to TSD rats and their controls.[7] As expected, $T_{ip}$ was increased in TSD rats and TSC rats. It did not differ appreciably between the two groups until it dropped below baseline in TSD rats on the last day of their survival. Also, selected ambient temperatures were not as high in the hyperthyroid TSD rats as they had been in the euthyroid TSD rats. On the other hand, survival time of hyperthyroid TSD rats was shortened by 37%.

How might high EE have contributed to the deaths of TSD rats? One possibility was the high metabolic rate per se, but this did not seem likely. Survival had not been lengthened in the hypothyroid TSD rats. Two control rats which were subjected to periodic disk rotation that produced only minor sleep loss were maintained in ambient temperatures of <10°C. Their EE was raised to about the level in the hyperthyroid rats. However, they survived until the experiment was terminated after 34 days—about twice as long as survival of euthyroid TSD rats and three times as long as survival of hyperthyroid TSD rats.

Other possible causes of shortened survival in hyperthyroid TSD rats are tissue breakdown or loss of energy reserves secondary to the high metabolic rate. These possibilities are supported by the Everson and Wehr report[26] of lengthened survival (to 25.5 days) of TSD rats which had minimal weight loss on a high calory diet. Although tissue loss or depletion of energy reserves might have contributed to mortal processes, they were not the necessary, decisive factors, since these rats died nevertheless. Also, food restricted rats which were not sleep deprived did not die until they had lost about twice as much body weight as TSD rats.[27] Although neither elevated metabolic rate, tissue loss, or depletion of energy reserves alone were responsible for the deaths of TSD rats, it is possible that an unidentified process normally reversed by sleep was accelerated by thyroxine administration.

Because hyperthyroid rats maintained $T_{ip}$ above baseline until just prior to death, because their survival times were shortened, and because survival time had not been decreased in hypothyroid rats in spite of their faster and further decline in $T_{ip}$, it is unlikely that hypothermia had been the cause of death of TSD rats. There were additional indications that, although hypothermia was a precursor of death, it was not the cause of death in TSD rats.[28] TSD rats whose brain temperatures were maintained near baseline by ambient heating died nevertheless. Survival time was not reduced in TSD rats maintained in cool ambient temperatures which lowered $T_{ip}$ over the whole course of deprivation.

The reduction of thyroid hormone levels in TSD rats had the unexpected effects of dramatically reducing the disheveled, yellowing fur, debilitated appearance, and skin lesions that had been so obvious in the untreated TSD rats. Throughout most of deprivation, the hypothyroid TSD rats looked like normal rats. It is unlikely that the skin lesions and appearance changes had been produced in TSD rats by high EE or thyroid hormones alone, since they had not been seen in rats subjected to the prolonged administration of thyroid hormones.[29] The physiological basis of the appearance changes and skin lesions in euthyroid TSD rats remains obscure. The skin lesions are particularly mysterious since the dermatologists who worked with us could not specify their cause, nor had they ever seen them described in any other condition. It is apparent, however, that the appearance and skin changes are not inevitable effects of sleep deprivation but depend on some interaction of sleep loss with elevated EE and/or thyroid hormones. Also, the excessive heat loss in sleep-deprived rats does not depend upon disruption of the fur or skin lesions on heat-dissipating surfaces.

## Temperature Regulation

The large magnitude of the thermoregulatory changes seen in the sleep deprived rats is evident more in the attempts to support or compensate for them than in the changes in brain and body

temperatures. $T_{ip}$ declined even though EE doubled. Near the end of deprivation, TSD rats chose initial ambient temperatures of more than 50°C (hot to the touch) in a thermal gradient and operantly maintained mean cage temperature at 37°C compared to a baseline mean of 26°C. One would have thought that the processes underlying such large effects might have been easily revealed, but that was not the case.

Since prostaglandins raise $T_{set}$, TSD rats were given aspirin (acetylsalicylic acid [ASA]), which blocks prostaglandin synthesis.[30] The characteristic rise in $T_{ip}$ at the beginning of TSD was reduced, but only by 26%. Because opiods may also increase $T_{set}$, we administered naltrexone, an opiod receptor antagonist, to TSD rats.[31] Again, there was little effect on the early $T_{ip}$ rise, Because the preoptic anterior hypothalamus (POAH) is a major brain center for thermoregulation, we lesioned this area to determine whether, under baseline conditions, the lesioned rats would show EE and $T_{ip}$ changes like those produced by TSD.[8] Although the lesions were sufficient to reduce the homeostatic regulatory responses to changes in ambient temperature, they produced no substantial changes in EE or $T_{ip}$ at the thermoneutral ambient temperatures which prevailed during the TSD procedures. Thus, it is unlikely that POAH impairment alone could have accounted for the thermoregulatory changes in sleep deprived rats. The POAH lesions did exacerbate the $T_{ip}$ declines in TSD rats, which suggests that the POAH normally defends against temperature declines in TSD rats that are produced by other, yet unidentified heat retention deficits.

Because vasoconstriction is a major defense against heat loss, we examined the effect of TSD on this defense. Normally, rats maintained in a cool (20°C) environment show decreases in $T_{ip}$ in response to the adrenergic antagonist phentolamine because it blocks vasoconstriction. TSD rats tested at 20°C also showed $T_{ip}$ and brain temperature decreases in response to phentolamine, indicating that noradrenergically mediated vasoconstrictors had been intact prior to phentolamine injection.[32] Late in deprivation, all TSD rats, when exposed to the cool ambient test temperature, showed a precipitous decline in body and brain temperature from which they did not recover, even when the test chamber was rewarmed—implying an exacerbation of the heat retention deficit and/or a failure of thermogenic compensation. In four of these rats, the thermoregulatory collapse occurred before phentolomine could be injected. The other six TSD rats had shown temperature declines in response to phentolomine prior to the thermoregulatory collapse—implying that adrenergic vasoconstrictor defenses had been working prior to the injection and that the collapse resulted from failure of mechanisms not adrenergically mediated.

To evaluate vasoconstrictor defenses independent of -adrenergic blockade, peripheral vascular resistance (PVR) was calculated from aortic blood pressure and blood flow at the aortic-illiac junction.[33] There was a small decline in PVR early in TSD which could have increased heat loss, but there was no evidence of a massive vasodilation which could, in itself, account for the large, progressive heat loss over the course of deprivation. There was, however, evidence of impaired vasoconstrictive defenses in TSD rats inasmuch as they showed lower PVR than control rats during most of deprivation even though the TSD rats were farther below $T_{set}$. A large increase in PVR at the start of recovery from TSD suggested a release from TSD-induced blockage of vasoconstrictive defenses. The results of both studies of vasoconstriction leave us in the somewhat awkward position of having demonstrated a failure of vasoconstrictive defenses against heat loss in later stages of TSD, but not knowing what was responsible for the original heat loss.

Since we have not identified the peripheral mechanism of the initial excessive heat loss, it possible that the routing of food energy to non-thermal forms might have made it difficult for sleep deprived rats to efficiently maintain body temperature in spite of huge increases in food intake? If so, we don't know what the alternative routing of energy might be. Food energy certainly did not go into storage (weight), it was not dissipated in locomotion (above control levels), it was not excessively dissipated in wastes, and loss of heat to the water under the disk was ruled out as a sufficient explanation of energy loss by yoked water exposure control rats[24] and by evidence of increased energy use in rats deprived of sleep by a subdermal thermal stimulation procedure which did not involve exposure to water.[34]

## Changes in the Brain

Widely held beliefs that sleep is homeostatically regulated by the brain, or that sleep is necessary for brain integrity, or that the effects of sleep deprivation are mediated by changes in the brain would suggest that sleep deprivation which was severe enough to cause major changes in metabolism, temperature regulation, brain-regulated hormones, and eventual death might be severe enough to produce obvious, profound changes in brain chemistry or morphology. Although some specific changes were seen, massive, profound changes were not found.

Several investigators have implicated monoamines in sleep regulation, but effects of sleep deprivation on brain monoamines have been varied and contradictory. We examined regional brain levels of serotonin, norepinephrine, dopamine, and their metabolites in rats subjected to TSD for 11-20 days.[15] No significant differences from yoked controls were found. These surprising results do not rule out a role for the monoamines in sleep regulation, but they raise doubt that they are functional targets of sleep or that their levels are sensitive to recent sleep history. To evaluate a theory that PS serves to upregulate noradrenergic receptors, we examined the effect of 10 days of PSD on regional brain levels of adrenoreceptors.[14] No significant PSD-control differences were found that could be unambiguously attributed to PS loss. Because acetylcholine is important for evoking PS, and because we found huge rebounds of PS following extended TSD, we evaluated the effect of 10 days of TSD and PSD on cholinergic receptors.[35] Nicotinic receptor binding was not affected in any of the several brain regions examined. There were a few small regionally specific changes in muscarinic receptor binding, but none in the pontine region most closely associated with the cholinergic stimulation of PS.

Earlier studies had not identified any morphological changes in body organs sufficient to explain the SDEs. Neither have morphological studies revealed dramatic, generalized damage to brain tissue which might readily explain the SDEs. Microscopic examination of cresyl violet and hematoxylin and eosin stained brain sections revealed no systematic differences between TSD and control rats in pathological manifestations.[36] Electron microscopic examination of seven brain areas revealed no differences between TSD rats and yoked controls in microtubule density.[37] Another electron microscopic study examined cell processes

(axons and dendrites) and organelles of the neuronal perikaryon (including mitochondria, Golgi apparatus, ribosomes, nucleoli, and lysomes) in three brain regions ( cortex, preoptic-anterior hypothalamus, and dorsal-lateral pons) of TSD rats, their yoked controls, and home cage controls.[38] No obvious group differences were found in either cell processes or perikaraya of any region. Neither were there any significant differences in synaptic density between groups. Another study[39] found no substantial evidence of brain cell degeneration as indicated by the TUNEL stain for DNA fragmentation, the Flouro-Jade stain for neural degeneration, or cortical mRNA levels of stress genes or apoptosis-related genes. One study did find significant amino-cupric-silver staining, which is highly sensitive to degenerative changes, in the supraoptic nucleus of the hypothalamus.[40] This result might somehow be related to the distinctively high rate of protein synthesis in this nucleus.

The histological studies reported above indicated that the SDEs did not result from any massive, generalized degeneration or death of brain tissue—which is consistent with the relatively rapid reversal of SDEs when recovery from TSD or PSD is permitted.[41] Accordingly, if the SDEs do result from brain pathology, then the causes are most likely to be found in very specific damage which has escaped us or in specific or generalized functional changes. In the search for functional impairment, we examined the effect of TSD on expression of the immediate early gene Egr-1 because it reflects synaptic excitation and might thereby be sensitive to either sleep-deprivation induced reductions of functional activity or sites of increased neural activity during sustained wakefulness.[42] TSD rats showed tendencies toward increased Egr-1 expression in 4 of the 25 brain regions examined (dorsal raphe, lateral habenula, superior colliculus, and ventral periaqueductal grey), but no differences from control rats in most regions or in whole brain Egr-1 mNRA.

Another search for TSD-induced functional changes in brain activity was led by Carol Everson, who has vigorously pursued the effect of DOW sleep deprivation in her own laboratories since graduating from our laboratory following the studies reported in the 1989 paper. Everson et al.[43] used cerebral energy metabolism, as measured by cerebral glucose utilization, as an indicator of functional brain activity. In spite of the large increase in systemic EE, average glucose utilization was unchanged in the brain as a whole and was not elevated in any of the 60 brain structures examined. Some regional decreases were found, most notably in hypothalamus, thalamus, and limbic system. Everson et al. have suggested, some correspondence between hypometabolic brain regions and some aspects of the SDEs (e.g., lowered glucose utilization in the preoptic nucleus of the hypothalamus which is involved in temperature regulation). However, Everson et al. did not propose that these regional declines in cerebral glucose utilization in and of themselves reflected brain impairment. The average hypothalamic decline in TSD rats relative to controls was only 12.8% compared to a decrease of about 30% in cerebral metabolism during normal NREM sleep in a study of monkeys[44] cited by Everson et al. This comparison suggests that the observed metabolic decreases in TSD rats might have reflected their sleepiness rather than brain impairments. If they reflected brain impairment, we would be faced with the strange conclusion that the brain is more impaired by normal sleep than by 11-12 days of sleep loss.

Summing up, we did not find changes in brain morphology or function sufficient to explain the dramatic systemic effects of sleep deprivation in the rat.

## Host Defense

One of the areas where there has been a major shift in our thinking is in host defense against invading pathogens. Our 1989 series included a paper[45] showing that standard measures of immune system patency, including splenocyte count, in vitro mitogen-induced lymphocyte proliferation, and in vitro and in vivo plaque formation in response to various antigens, for both TSD and PSD rats did not substantially differ from their yoked and cage control values. Because these results do not support the hypothesis that sleep deprivation results in immune suppression, our interest in host defense (of which the immune system is a major component) disappeared. Then, in 1993, Everson[46] reported bacteria in the blood of five of six near-terminal TSD rats, but none in their yoked controls, indicating TSD-induced failure of host defense, an indication we subsequently confirmed.[9] Everson's finding raised several questions: What is the source of the host defense failure? Does this failure mediate other SDEs? Does the death of TSD rats result from host defense failure?

**Source of host defense failure:** Two major components of host defense are physical barriers (skin, the lining of the gut, cilia in the airways) and the immune system. We have proposed that the major flaw is in the barriers, specifically that the lining of the gut becomes porous, allowing translocation of bacteria from the gut to the peritoneal cavity.[9] Everson and Toth[47] have also proposed translocation as a route of bacterial infection in TSD rats, but they have also argued for a generalized failure of immune function as indicated by the lack of inflammation around the skin lesions and the absence of fever. They discounted our early report of intact immune function in sleep deprived rats because about half the rats showed reduced and half showed increased lymphocyte responsiveness, thereby indicating "inconsistent effects."[48] This reasoning fails to recognize that there was no mean degradation of immune function as would be expected from their position.[49]

Although the evidence for translocation is weak,[9] the evidence against the other options, particularly immune failure, is strong. In addition to our 1989 paper, we also showed that when rats are injected with allogenic tumor cells, the resulting tumors grew less and regressed more rapidly in TSD rats than in yoked controls[17] The time course was typical for a T lymphocyte-mediated response. Thus immune response in this case was enhanced, a not-uncommon result (reviewed in reference[17]).

**Failure of host defense as a mediator of SDEs:** Everson has suggested that other SDEs might be secondary to host defense failure. To test whether this is the case, we blocked invasion by gut bacteria, using an orally administered cocktail of broad-spectrum antibiotics to kill all aerobic and facultatively anaerobic bacteria in the gut.[9] (When we found no bacteria in the gut, no bacteria were found in the blood, liver, kidney, or mesenteric nodes—a major argument for the gut wall as the primary site of host defense failure.) After protecting against bacterial invasion, we looked for SDEs either early in TSD (day 4) or late (when $T_{ip}$ had dropped below baseline). After four days, TSD, "clean" rats still had elevated $T_{ip}$ and EE, and nascent skin lesions. Dosing the rats late in deprivation did not lower EE, reverse the skin lesions, or raise $T_{ip}$, even temporarily. Although

it is possible that the "clean" rats were all suffering from systemic infection by virus, obligatory anaerobes, or eukaryotic pathogens (none of which we attempted to detect) or by undetected aerobes, given that most host defense failures had involved aerobes which were readily detected, it is unlikely that host defense failure contributed much to the SDEs.

**Failure of host defense as a factor in survival:** It would seem obvious that even if failure of host defense did not have a great effect on the other SDEs, it might still play a deciding role in how long the rats survived. However, the rats given antibiotic cocktails near the end of deprivation continued their temperature decline and died "on schedule" even though no trace of systemic infection was found.

## OVERVIEW

The two authors of this paper are now retired; all the graduate students who worked so hard and faithfully on this research have completed their doctoral degrees and have left for various research and teaching positions; we have closed our laboratory. The future of the kind of research we have described here is now in other hands. The most vigorous pursuits of sleep function using the DOW method are now being conducted by Carol Everson, who is working on physiological effects of TSD at the Medical College of Wisconsin in Milwaukee, and by Chiara Cirelli and Guilio Tononi, who are working on the effects of TSD on gene expression and energy conversion at the University of Wisconsin. New studies with the DOW method are being undertaken by Ling-Ling Tsai at the National Chung-Cheng University in Taiwan and by Paul Shaw at the Neurological Institute in San Diego. Ruth Benca, Ping-Fu Feng, Clete Kushida, Carol Landis, William Obermeyer, and June Pilcher continue to do sleep research in other laboratories using other techniques. Marcia Gilliland and Carol Zenko remain at the University of Chicago in research-administrative positions, Samuel Refetoff continues his long careers as our personal friend and Professor of Medicine at the University of Chicago. We think of them all with affection and wish them all the best of luck.

We think that the major impacts of our DOW sleep deprivation studies have been the demonstration by controlled experiments that, at least in the rat, sleep and paradoxical sleep are biological necessities and that extended sleep loss reliably produces a syndrome of specific, substantial physiological changes. Certainly, we did not discover anywhere near as much as we would have liked about why sleep is necessary. The deaths of the sleep deprived rats were the most dramatic consequences of sleep deprivation, but since death per se is such a nonspecific symptom, and since we did not find an unambiguous cause of death, that dramatic symptom did not tell us much about why sleep was necessary. Perhaps the most promising leads to functional significance are the three thermoregulatory failures. They are large, reliably elicited, functionally important changes worthy of further study. We have no clear idea of how that search should be pursued. Ideally, it would be interesting to record individual thermoregulatory neurons and observe how their spontaneous and responsive firing patterns change with sleep and extended sleep deprivation. A major problem would be how to hold such neurons over a long course of sleep deprivation.

Thus far, we have not had much success in deciphering how the thermoregulatory effects of sleep loss are mediated. Similarly, the cause of death, the deterioration of appearance, the skin lesions, and how brain activity is changed have resisted explication. As unlikely as it might seem, perhaps the problem is that the mediation might be related to functionally significant physiological processes that have not yet been clearly identified—and that may be why the function of sleep has itself been such a tough nut to crack.[50]

Another possibility is that at least some of the SDEs have multiple mediators. The increase in EE, for example, may be the sum of the cost of maintaining an elevated $T_{set}$ plus the cost of Waking per se, plus the energy cost of water exposure under conditions of mildly impaired vasoconstriction. Similarly, sleep deprivation may cause death by enhancing the rat's exposure to multiple potentially life-threatening conditions including systemic infection, hypothermia, and malnutrition. Then, if one condition is blocked, death will occur by an alternate route.

A major problem with our research on the effects of sleep deprivation in that rat is that we don't have a firmly fixed picture of how specific the effects may be to the rat. Sleep is so ubiquitous among mammals that it is difficult to imagine that it has very different functions among different species, thereby producing different functional deficits when sleep is prevented. On the other hand the evidence on the generality of the SDEs across species is quite mixed, as indicated by our review in the 1989 paper. Part of the problem is the difficulty in enforcing sleep deprivation in other species for as long as we have done it with the DOW method in the rat. Unfortunately, the DOW method was designed for the rat and is relatively restricted to animals of about the same size that would undergo severe frustration and physiological change before they would go to sleep while lying in water 2 cm deep. (Mice are probably too small to permit a firm enough attachment of the head plug to the skull to withstand many days of recording which permits free locomotion.) Successful comparisons of results across species will have to rely more on similar measurements of effects than on similarity of recording methods.

One potential use of the DOW method that has not been exploited is in the study of the effects of PSD on performance, learning, and memory. PSD for a few days requires relatively few disk rotations and is methodologically clean compared to the confounds of the widely used "flower pot" procedure.

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