Sustained sleep deprivation impairs host defense

CAROL A. EVERSON
Clinical Psychology Branch, National Institute of Mental Health, Bethesda, Maryland 20892

Everson, Carol A. Sustained sleep deprivation impairs host defense. Am. J. Physiol. 265 (Regulatory Integrative Comp. Physiol. 34): R1148–R1154, 1993.—Prolonged sleep deprivation in rats causes an unexplained hypercatabolic state, secondary malnutrition symptoms, and mortality. The nature of the vital impairment has long been a mystery. Its determination would help to elucidate the type of organic dysfunction that sleep prevents. There are no gross detectable disturbances in intermediary metabolism, clinical chemistry, or hematological indexes that provide substantial clues to the mediation of sleep-deprivation effects. Furthermore, postmortem examinations reveal no systematic morphological or histopathological findings. Taken together, the cachexia and the absence of evidence of structural damage or organ dysfunction pointed to involvement of a regulatory system that was diffuse, possibly the immune system. Blood cultures revealed invasion by opportunistic microorganisms to which there was no febrile response. These results suggest that the life-threatening condition of prolonged sleep deprivation is a breakdown of host defense against indigenous and pathogenic microorganisms.

infectious disease; fever; metabolism; bacteremia; septicemia; immune system

SUSTAINED SLEEP DEPRIVATION in humans and laboratory rats is lethal. Humans suffering from fatal familial insomnia, a genetically transmitted prion disease, die 7–25 mo after the onset of progressive and untreatable insomnia caused by degeneration of thalamic nuclei (19, 21). Correlational studies in adult and elderly humans show strong relationships between sleep amount and longevity (16, 27). Sustained sleep deprivation in the laboratory rat causes a hypercatabolic state that ends in death after an average of 9 days (9, 30). The animals’ response to prolonged sleep deprivation is marked by two phases: a chronic phase characterized by progressive increases in food intake, losses of body weight, and development of secondary malnutrition symptoms (9, 12), followed by an acute phase lasting one to several days marked by a wasted appearance and hypothermia while energy expenditure is very high (9, 10). Despite sleep’s vital nature, no critical biological impairment induced by sleep deprivation, even lasting many days, has been found to explain the pathogenesis or mortality (29). Many plausible explanations of the hypercatabolic state in rats have been ruled out; among them, diabetes (9), malabsorption of calories (2), changes in total body water (2), locomotor activity (2), behavioral distress (29), and gross perturbations of intermediary metabolic pathways of glucose, fat, or protein (9). Also, plasma ACTH and corticosteroid levels increase appropriately during stress challenge tests, indicating sufficiency of the adrenal axis (8). To increase the likelihood of identifying a dysfunction that could then be examined to determine how the pathology unfolds, research efforts in the present study were focused on the latter cachetic phase that precedes death by only a few days. Determination of even one predisposing factor that eventuates in death would help elucidate the biological consequences of sleep deprivation and substantiate a physiological role for sleep.

The specific aim of the present experiment was to determine whether sleep-deprived rats develop bacteremia. Five features of the clinicopathological profile discussed below resemble the deleterious effects of toxic biochemical factors, rather than structural damage, and an affected regulatory system that is diffuse, such as the immune system. First, examinations of body tissues for the presence of morphological changes have not revealed common internal lesion foci, hemorrhages, inflammation, or other indications of vital organ impairment; loss of white adipose tissue and loss of the fatty appearance of connective tissues and membranes are the only findings (9). [Erosions of the stomach mucosa are found when the animal appears to be near death and has no food in its stomach (Ref. 29 and unpublished observations).] Histopathological examination of organs and glands, including the brain, reveals no systematic change as compared with controls (13). Thus, despite a distinctive malignant course, structural damage has not been found and the pathogenesis appears to be generalized or has the multifarious effects expected of a toxemic state. In addition, there are no seizures, convulsions, or diarrhea that might indicate greater involvement of some organ systems relative to others. Even in patients that died from fatal familial insomnia, indications of organic impairment have not been remarkable beyond the thalamic degeneration that appears to have caused the insomnia; when complete autopsies were performed, the findings were limited to bronchopneumonia and various degrees of cardiac hypertrophy (19). The second reason that toxic factors were suspected is that resumption of sleep in the rat completely reverses all observed sleep deprivation-induced changes in metabolism and plasma hormone concentrations within a few days, even when debilitation is severe (10). Recovery would not be expected quickly if the primary pathogenesis were neuronal degeneration or primary organ failure. Bacterial infections and toxicity are two of the few conditions that predispose to death, and yet can be readily reversible without permanent impairments to regulatory functions. Third, the unexplained catabolic phase induced by sleep deprivation shares several features with a chronic septic challenge (reviewed in Refs. 7, 28, 31, 33–35). These include increased heart rate (9); progressive increases in energy expenditure (9); early skin lesions (18); progressive elevations in plasma norepinephrine (2), alkaline phosphatase (11), and blood leukocytes (12); suppressed plasma thyroxine and triiodothyronine (2); late, mild increases in corticosteroids that mirror declining body temperature (2); moribund elevations in plasma glucose and lactate dehydrogenase (12); and normal urine (9). Fourth, the terminal phase in the sleep-deprived rat resembles septic shock with multiple organ
system failure. Before collapse, a decline in core temperature is accompanied by lethargy, aphagia, adipisia, paw edema, wasted appearance, and decreased waking electroencephalogram (EEG) amplitude (9, 30). And lastly, microbial diseases and associated immune system responses lack strong prognostic indicators and yet are highly lethal (6). In the same way, a proportion of chronically sleep-deprived rats that are not distinguishable from survivors never attain substantial rebound sleep when permitted and continue to worsen and die (8). In fact, the only known cure for the deprivation-induced debilitation is sleep, though a point is reached when even permitting sleep cannot prevent death.

An initial attempt to assess immunocompetence in sleep-deprived rats, by measurement of in vitro splenic B and T cell proliferation responses to mitogens and B cell production of antibodies, failed to find an impairment (1a). However, because of these many factors, i.e., the diffuse, nonlocalized, and toxic-like nature of the pathogenesis, the marked cachexia, and the quick reversibility of debilitation (with sleep) without evidence of permanent damage, the most plausible cause of death from sustained sleep deprivation is septicemia. In the present study, heart blood of sleep-deprived rats and controls was cultured to determine whether viable bacteria were present. Also, core temperature was assessed to detect expected febrile responses to systemic infection, and tissues were examined for evidence of inflammatory reactions.

The results indicate that sustained wakefulness eventuates in bacteremia and a septicemic death. Furthermore, there was no febrile response and poor tissue inflammatory reactions to the systemic infection, which suggest an immunosuppressed state.

METHODS

Subjects and surgery. Animal care and use were in accordance with the National Institutes of Health guidelines, and the experiment was conducted under a protocol approved by the National Institute of Mental Health Animal Care and Use Committee.

Each of six experimental runs (experiments 1–6) was composed of a set of procedures on two adult male rats that served as sleep-deprived and yoked-control subjects. Four of these experimental runs included a third rat that served as a surgical control. The rats were Sprague-Dawley (Harlan and Zivic-Miller), weighed 513 ± 58 (SD) g, and were 22–28 wk old. Surgery was performed under deep anesthesia and analgesia (ketamine HCl, 100 mg/kg ip; xylazine HCl, 2.4 mg/kg im; atropine sulfate, 0.1 mg/kg im; 1–2% lidocaine HCl, 2.4 mg/kg sc abdominal incision site, and supplemental doses of ketamine HCl, 10 mg/kg ip as needed for state maintenance). To determine vigilance state for automated sleep deprivation procedures and to quantify wakefulness and sleep stages, each animal was implanted with microelectrodes for recording of cortical EEG and hippocampal theta, and temporalis electromyogram (4). For continuous recording of core body temperature, a telemetric transmitter (Barrows) was implanted in the peritoneum. For withdrawal of blood samples under the freely moving conditions of the experiment, animals in the first two experimental runs were implanted with jugular catheters. Catheter lumens were kept clean with daily flushes of sterile heparinized saline containing ampicillin, 1.25% (wt/vol).

Sleep deprivation and yoked-control procedure. Prolonged sleep deprivation was accomplished by a yoked-control paradigm that has been shown to be highly selective for the deprivation of sleep without interfering with normal waking activities (6, 12). In brief, after 7 days of recovery from surgery, the two rats designated to be either sleep deprived or yoked control completed 7 days of baseline measurements housed in an apparatus on either side of a large, round, horizontal platform (46 cm diam) that was divided in half. Beneath this platform at a distance of 3 cm was water that was 2 cm deep. Although the rats were free to step into, sit in, and walk around in the water in their half of the apparatus at any time, both spontaneously preferred the platform where they were also unrestricted in movement but could sleep and where food was easily accessible.

After the baseline period, one rat was sleep deprived by automatic rotation of the housing platform whenever changes in amplitude of EEG, electromyogram, and theta signals indicated sleep onset. The disk rotated slowly and passed beneath the dividing cage wall, and because of their preference to remain on the platform and to avoid the water both rats would walk in a direction opposite to the rotation. The water had the properties of a soft boundary to the periphery of the platform and served as an avoidance stimulus that did not have to be increased in intensity or duration. The amount of applied forced locomotion was minimal, because each rotation was 6 s and moved the platform one-third revolution; the platform was then motionless until sleep onset was again detected in the sleep-deprived rat. The yoked control could sleep when the experimental rat was in a wakefulness bout (typically engaged in eating, grooming, or exploratory behavior) because no contingencies were in effect and the platform was still. If, however, the yoked control was asleep when the experimental rat fell asleep, it was also aroused because of the platform movement. In this way, the yoked control served as a comparison for the experimental environment and the timing, frequency, and duration of the forced locomotion. The third, surgical-control rat served as a second control for possible contamination of the bloodstream from microbes associated with surgical implants and other sources. It was housed in a cylindrical cage (30 cm OD × 30 cm high) attached to the same recording cable arrangements as were the experimental and yoked subjects in the apparatus.

Food intake and body weight were monitored daily throughout the baseline and experimental periods for all groups. Rats were fed ad libitum with a balanced, purified diet that was augmented with protein and isocaloric to normal (40.7% protein, 44.7% carbohydrate, 4.3% fat, and 10.3% minerals, fiber, and vitamins), which was previously found to produce less variability in survival time of sleep-deprived rats (12).

Body temperature. Signals emitted from the peritoneal transmitters were digitized and timed by a microprocessor (12), which provided a measurement of body temperature every 30 s. The presence of fever was assessed by a comparison of both the absolute temperature maxima and the temperature fluctuation around the daily mean between the normal, baseline period and the experimental period. The maximal temperature during a given experimental day was defined as the highest temperature that was sustained or exceeded for two consecutive minutes. For fever to be present during the experimental period it was necessary that some normal range be exceeded. The normal, expected temperature variation was derived from measurements during the baseline period, and the resultant average temperature maxima across rats was 1.3°C above the daily mean. During the experimental period, a febrile spike was considered to be present if the daily maximal temperature exceeded the normal maximal temperature variation by 1°C.

Blood specimen collection and analysis. Heart blood specimens were taken and necropsy examinations were initiated when the average daily core temperature of the sleep-deprived rat was >1°C below baseline, concomitant with an increasingly
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Table 1. Wakefulness and sleep stage percentages

<table>
<thead>
<tr>
<th>Group/Condition</th>
<th>n</th>
<th>Wakefulness</th>
<th>TL Sleep</th>
<th>TL NREM</th>
<th>HS</th>
<th>LS</th>
<th>PS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep deprived</td>
<td>6</td>
<td>46.7±1.3</td>
<td>53.3±1.3</td>
<td>48.1±1.7</td>
<td>43.5±1.8</td>
<td>4.5±0.3</td>
<td>5.3±0.8</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td></td>
<td>90.3±1.0</td>
<td>9.7±1.0</td>
<td>8.9±0.6</td>
<td>4.6±0.8</td>
<td>4.5±0.1</td>
<td>0.8±0.4</td>
</tr>
<tr>
<td>Yoked control</td>
<td>6</td>
<td>46.4±2.7</td>
<td>53.6±2.7</td>
<td>47.8±2.7</td>
<td>43.3±2.7</td>
<td>4.5±0.1</td>
<td>5.8±0.6</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>68.0±4.5</td>
<td>41.4±4.5</td>
<td>38.3±4.6</td>
<td>34.0±4.7</td>
<td>4.3±0.1</td>
<td>3.1±0.4</td>
</tr>
</tbody>
</table>

Values are means ± SD (n=1); n = no. of subjects. TL, total; NREM, non-rapid eye movement sleep; HS, high EEG amplitude sleep; LS, low-amplitude sleep; PS, paradoxical (i.e., rapid eye movement) sleep. Total sleep is composed of NREM (i.e., HS and LS) and FS.

RESULTS

Sleep-deprived rats were awake 90 ± 1.0% (SD) of total time and yoked controls were awake 59 ± 4.5% (SD) of total time. Table 1 shows the sleep stage percentages of total time during baseline and experimental periods for sleep-deprived and yoked-control groups.

Criteria used to indicate the presence of a pathological, life-threatened state included a febrile appearance and a drop in core temperature to >1°C below baseline. The number of days of sleep deprivation required to meet these criteria are shown in Table 2. Core temperature declines to >1°C had not been met the day before and serious hypothermia could happen precipitously, as indicated by the final 4-h temperature measurements shown in Table 2. The telemetric transmitter failed in the sleep-deprived rat of experiment 4, and the decision to conclude the experimental run was based on the rat's appearance, low 24-h food and water intake, and accelerated weight loss. Rectal temperature in this rat before administration of anesthesia was 29°C. Even though lethargy and apparent weakness supervened on a general deteriorated appearance within the last 24 h, which corresponded with meeting temperature criterion for necropsy, all sleep-deprived rats maintained an upright posture and walked in response to the arousal stimulus, appeared oriented with intact sensorium, and showed no gross signs of myopathy or neuropathy.

Viable bacteria were present in the heart blood of five of six sleep-deprived rats and none of the six yoked (experiments 1–6) and four surgical-control (experiments 3–6) rats. Species cultured are shown in Table 1. All organisms were opportunistic and facultative anaerobes of both gram-negative rod and gram-positive cocci classifications. The possibility that this result was due to contamination is greatly limited by the fact that specimens obtained by the same aseptic procedures were coded before cultures were made, and yet, after decoding, all positive cultures belonged to only the sleep-deprived group. Each of the five sleep-deprived rats had at least one microbe recognized for its highly lethal effects once in the bloodstream of an immunocompromised host. The...
result of negative cultures for the sixth sleep-deprived rat (experiment 5) is equivocal for bacteremia. Given the near-identical clinicopathological course of all sleep-deprived rats, it is possible that a pathogen might have been present that was not detected because of the single blood sample trial. Factors affecting successful recovery of microbes include the broth medium, specimen dilution, the timing of the sampling period relative to intermittent bacteremia, and unidentified nonbacterial pathogens that can cause similar host reactions (1).

A profound catabolic state in sleep-deprived rats was manifested by high food intake and weight loss (Table 1). Four of six sleep-deprived rats had food intake levels 55 to 238% above baseline during the final 24-h period. The other two sleep-deprived rats had food intake levels 45 and 84% above baseline the previous day, and this declined abruptly to 35 and 56% below baseline amounts, respectively, during the 24 h before necropsy procedures. Despite the high food intake, sleep-deprived rats lost 8–30% of their baseline body weights. Although the sleep-deprived rat of experiment 5, for which cultures were negative, was cachectic and had met necropsy criteria, it had the highest food intake during the final 24 h. High food intake has been previously shown to be adaptive (9, 12) and whether this is an important influence on the prevalence of bacteremia in sleep-deprived rats is yet to be resolved through future studies. In contrast, the drop of food intake in two of the other sleep-deprived rats during the 24-h period before examination was an indication of the onset of aphagia that eventually occurs (3), which is also characteristic of experimentally induced septicemia (33).

Despite bacteremia, no febrile spikes were observed throughout the experimental period for any sleep-deprived or yoked-control rat; nor was shivering observed. The daily fluctuations in core temperature and the time to hypothermia are shown in Fig. 1. Even in the worst case, the maximal temperature for even one 24-h experimental day before the steep negative slope of the last day did not exceed an expected, ultradian maximum by more than 0.3°C for any sleep-deprived rat, comparable to 0.4°C for any yoked-control rats (except for the yoked-control rat of experiment 2 that had occasional, low daily means and, therefore, temperature swings that were larger than usual).

Internal examination of the animals did not show a marked host response to the systemic infection. Erosions of the stomach mucosa and translucent intestinal walls distended with gas in places were found in the two sleep-deprived rats (experiments 4 and 6) that had decreased food intake and had no food in their stomachs upon examination; this was not an unexpected result of the more advanced morbidity in these two rats. Histological examinations revealed many minimal to mild lesions of specific organs that were not distinguishable from background variation. These included minimal to mild histiocytosis or hemorrhage of the lungs (common in Sprague-Dawley adult rats), minimal to mild, chronic nephropathy (common in adult rats), minimal congestion of the spleen (yoked-control rat, experiment 4), minimal degeneration and mild necrosis of the adrenal cortex (sleep-deprived rat, experiment 6 and surgical-control rat, experiment 5). Moderate (i.e., beyond background variation) inflammatory lesions of the lung (interstitium) were found in one yoked-control (experiment 4) and one surgical control (experiment 6) rat. Findings consistent with bacterial infection included heart lesions (minimal, subacute inflammation of the adventitia of the aorta) in the sleep-deprived rat of experiment 6 and changes in the spleen (mild to moderate granular pigment denoting hemosiderin and minimal to mild follicular atrophy consistent with debilitation) of sleep-deprived rats of experiments 3, 4, and 6. The brain, liver, small intestine, colon, and mesenteric lymph nodes were normal. Acute inflammation and hyperplasia of the popliteal lymph node were found in sleep-deprived rats of experiments 5 and 6, which was consistent with skin lesions on extremities (discussed below).

Because severe pathogenic foci were not found upon
internal examination, the suspected portal of bacteremia entry into the bloodstream was the characteristic and stereotypic lesions that develop on the skin of sleep-deprived rats. These have previously been described and characterized histologically (18). A few skin lesions developed on four of six yoked-control (and no surgical-control) rats, but they did not approach the severity of those of sleep-deprived rats at the time of final examination.

DISCUSSION

The nature of the life-threatening state of sustained sleep deprivation appears to be a breakdown of host defense mechanisms against opportunistic and pathogenic microorganisms that are in the environmental milieu. Systemic infection was associated with skin manifestations but not a febrile response or many significant tissue inflammatory reactions, which suggests an immunosuppressed state. Bacteremia did not appear to be merely an incidental accompaniment to death processes (and therefore to be expected) because the sleep-deprived rats were not incapacitated; e.g., they had consumed food within the 24 h before blood sampling. That the systemic infection was pathogenic is indicated by its close association with the critical end-point marker, death, and the fact that the invading microbes identified are known to be highly lethal. Taken together, the systemic infection associated with cachexia and an increasingly debilitated state suggest that death is due to septicemia.

Although tissue inflammatory reactions need not be marked to be meaningful, sleep-deprived rats appeared to have had a poor response compared with that typically found in most infectious disease states. Furthermore, tissue changes were not likely to have become greater with time if the experiment had been continued to the late moribund period; a previous study found no positive findings in grouped tissues of sleep-deprived rats that had died or were near death (13). Even the skin lesions, once they had developed from papules, did not appear swollen or inflamed.

Cause and effect between cutaneous manifestations and systemic infection can be in either direction, and the development of erythematous papules on the skin (including the non-weight-bearing aspects of the dorsal tail) might represent the first overt signs of a primary alteration in the general susceptibility of the host to pathogens (reviewed in Ref. 34), as well as a likely portal of entry. (Other explanations of portal of entry, e.g., changes in gut permeability or surgical and electrode sites have not yet been ruled out, but the microbes identified are often found on the skin.) Papules appear early, after 2–14 days of sleep deprivation, and become progressively enlarged well-circumscribed necrotic lesions that share features with ecthyma granulosa of Pseudomonas aeruginosa infection and other bacterial related dermatoses (34). Previous studies have found that the lesions cannot be accounted for by mechanical variables and water exposure per se (9, 12, 18). Although the initial occurrence of erythematous papules might suggest that the skin barrier has been primarily compromised by other factors (e.g., whole body catabolism), which results in secondary, localized infection sites, these would not be expected to cause a cascade of deleterious and lethal effects without other existing host defense impairments; e.g., poor inflammatory responses.

An afebrile state was maintained despite systemic infection even though there are indications that the central thermoregulatory set-point is elevated in sleep-deprived rats (24), as would be expected in a febrile state. Processes related to bacteremia can potentially explain the paradoxical effects of hypothermia during a hypercatabolic state while the central temperature set point is elevated. Hypothermia attributable to bacteremia is associated with different hemodynamic changes than controlled hypothermia or hypothermia caused by climatic exposure or drugs, whereas other clinical parameters (e.g., temperature, leukocyte count) do not distinguish between these groups (23). In bacteremia-related hypothermia, a high cardiac index and a low systemic vascular resistance suggest that heat loss is due to impaired vasoconstriction (23). Others have found that fever has survival value, and when not present during infection the outcome is poor (15, 23). Thus a febrile response is not diagnostic of bacterial infection in the severely sleep-deprived rat, and severe hypothermia may be attributable to an infection-related failure of heat retention mechanisms.

Whether processes related to impaired host defense are early or late mediators of the pathophysiology of sleep deprivation is yet to be determined and is under study. Impaired host defense might occur late in the malignant course of sleep deprivation. Hence, the unexplained catabolic state induced by sleep deprivation may rob energy from vital processes, resulting in a compromised and malnourished animal that is a prime candidate for endogenous bacterial challenge. Or, if excessive heat loss is indicated by early increases in whole body energy expenditure without an increase in body temperature, as others have suggested (24), the sleep-deprived rat might not be able to mount a fever response. On the other hand, it is likely that changes in host defense induced by sleep deprivation developed over days or weeks to culminate in a bacteremic state and, therefore, changes in immune parameters might be reflected in earlier pathophysiological effects. For example, challenges to immune integrity are expected to induce responses from cytokines (e.g., tumor necrosis factor, interleukins) known for their highly catabolic effects and their cascade of deleterious effects (reviewed in Refs. 33, 36) that could be mediators of earlier sleep deprivation-induced hypermetabolism.

In conclusion, the present finding of bacteremia in sleep-deprived rats provides a likely explanation of the cause of late cachexia, hypothermia, and mortality and establishes a starting place for determination of an impaired mechanism(s); e.g., neutrophilic dysfunction and/or impaired inflammatory responses. Of equal importance is the establishment of critical end point markers that will likely give meaning to earlier, perhaps non-pathological, changes in immune modulators for which it is otherwise difficult to establish clinical significance.

Perspectives

A role of sleep in preserving immunocompetence has long been suspected and has been supported previously by strong associative relationships between changes in sleep
and immune function parameters (5, 17, 22, 25, 26, 32). Also, bacteria, bacterial products, and immune system regulators can have potent somnogenic actions (reviewed in Refs. 14, 17). The present results indicate that the deprivation of sleep impairs whole organism host defense: without the experimental administration of any agent except sleep loss, prolonged wakefulness produced a life-threatening systemic infection that was not accompanied by the usual diagnostic symptoms of fever and large tissue inflammatory reactions. Therefore, these findings support the speculation that sleep has a role in immune function.

The present findings might be of immediate clinical relevance to humans, particularly the critically and terminally ill who may suffer profound sleep disruption because of pain, grief, discomfort, and therapeutic interventions. The present findings showed that sleep deprivation induces cachexia and impaired host defense in an animal without preexisting health impairment, and by extension it is possible that sleep loss may exacerbate, compound, or even underlie these same symptoms when health is compromised by other disease. Sleep deficiency, bacteremia, and their interaction might be nonspecific accompaniments of a deteriorated physiological condition, but they might also be decisive factors that could mean the difference between life and death.

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Address for reprint requests: C. A. Everson, Clinical Psychobiology Branch, National Institute of Mental Health, Bldg. 31, Rm 3536B, 9000 Rockville Pike, Bethesda, MD 20892.

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