Research report

Functional consequences of sustained sleep deprivation in the rat

Carol A. Everson*

Clinical Psychobiology Branch, The National Institutes of Health, National Institute of Mental Health, Building 10, Room 4S239, 9000 Rockville Pike, Bethesda, MD 20892, USA

Abstract

Sleep deprivation disrupts vital biological processes that are necessary for cognitive ability and physical health, but the physiological changes that underlie these outward effects are largely unknown. The purpose of the present studies in the laboratory rat is to prolong sleep deprivation to delineate the pathophysiology and to determine its mediation. In the rat, the course of prolonged sleep deprivation has a syndromic nature and eventuates in a life-threatening state. An early and central symptom of sleep deprivation is a progressive increase in peripheral energy expenditure to nearly double normal levels. An attempt to alleviate this negative energy balance by feeding rats a balanced diet that is high in its efficiency of utilization prolongs survival and attenuates or delays development of malnutrition-like symptoms, indicating that several symptoms can be manipulated to some extent by energy and nutrient consumption. Most changes in neuroendocrine parameters appear to be responses to metabolic demands, such as increased plasma catecholamines indicating sympathetic activation. Plasma total thyroid hormones, however, decline to severely low levels; a metabolic complication that is associated with other sleep deprivation-induced symptoms, such as a decline in body temperature to hypothermic levels despite increased energy expenditure. Metabolic mapping of the brain revealed a dissociation between the energy metabolism of the brain and that of the body. Sleep deprivation's effects on cerebral structures are heterogenous and unidirectional toward decreased functional activity. The hypometabolic brain structures are concentrated in the hypothalamus, thalamus and limbic system, whereas few regions in the rest of the brain and none in the medulla, are affected. Correspondence can be found between some of the affected cerebral structures and several of the peripheral symptoms, such as hyperphagia and possible heat retention problems. The factor predisposing to mortality is a decreased resistance to infection. Lethal opportunistic organisms are permitted to infect the bloodstream, which presumably results in a cascade of toxic-like reactions. Host defense is thus the first system to fail. There is neither fever nor marked tissue inflammatory reactions typical of infectious disease states, suggesting that sleep deprivation is immunosuppressive. Each of the four major abnormalities identified — (1) a deep negative energy balance and associated malnutrition; (2) heterogeneous decreases in cerebral function; (3) low thyroid hormone concentrations; and (4) decreased resistance to infection – can be viewed as having an early origin during the sleep deprivation process to signify the foremost pathogenic situation to which the other abnormalities might be secondarily related. The findings therefore remain somewhat equivocal for a unitary function for sleep, but can support putative roles for sleep in thermoregulation, energy conservation, immune system integrity and tissue restoration.

Key words: Sleep deprivation; Metabolism; Nutrition; Thyroxine; Cerebral glucose utilization; Thermoregulation; Host defense; Immune function

1. Introduction

There is general agreement that it is unhealthy, if not dangerous, to go too long without sleep; sleep deprivation impairs cognitive performance [36] and is associated with shortened longevity [40] and chronic sleep disturbances are health risk factors that appear to compound disease processes [50]. Still, little is known about how sleep deprivation affects the physiological functions of the brain and the body.

* Corresponding author. Fax: + 1 (301) 496-5439; e-mail: ceverson@pop.nih.gov

The results of short-term sleep deprivation in animals and humans have not yet pointed to physiological changes with clinical significance, even though deviations from normal can be found on some parameters. The most notable short-term changes are behavioral irritability and poor performance [36], but physical health does not seem to be compromised. If sleep serves important biological functions, it is not surprising that short-term deprivation has no serious consequences. The same is true of short-term deprivation of other basic requirements, such as food and water. For this reason, short-term deprivation may not yield many clues about the importance of the missing requirement or the consequences of long-term disruption.

Much is now known about the consequences of other deprivation states, such as nutritional deficiencies, which have been shown to have important roles not only in growth failure and death, but far-reaching and subtle roles in health, such as heart disease. The same scope of understanding is not yet true of the consequences of sleep deprivation.

The findings discussed in this paper are based on the assumption that subtle biological changes underlie the early effects of sleep deprivation (such as poor cognitive performance in humans), but that at this stage in our understanding, it is necessary to prolong sleep deprivation to identify the organic changes that develop and to investigate their mediation. Each type of basic deprivation manifests itself differently and evokes different homeostatic mechanisms in compensation for the missed requirement; e.g., the pathophysiology of dehydration is different from that of starvation or cold exposure. Sleep deprivation, too, appears to result in a unique clinicopathological profile.

2. Background findings of the consequences of prolonged sleep deprivation in laboratory rats

Prolonged sleep deprivation under carefully controlled experimental conditions is lethal to rats, whereas control rats yoked to the experimental conditions remain healthy. A 1983 report by Rechtschaffen and colleagues [60] showed that the lethality could not be accounted for by circumstances other than the sleep loss itself, thus establishing that sleep is indispensable. The length of the deprivation period required to reach a moribund state was 16 days (an average of 21 days in more recent studies [27,52]), compared with 17–19 days for food deprivation [23,27] and slightly longer for water deprivation [13]. Therefore, the rate at which the effects of sleep deprivation become manifest is similar to that for other basic deprivations. Subsequent studies of prolonged sleep deprivation in rats delineated the clinical parameters that seemed to be primarily affected by sleep deprivation and those that appeared relatively unaffected [7,9,10,27,28,41,59]. The experimental design used to deprive one rat of sleep while maintaining a yoked-control rat comparison and the discussion of evidence regarding the specificity of the effects (due to sleep deprivation rather than to variables inherent in the design per se), are described elsewhere [9,10,27,31, 41,59].

The earliest, most consistent and robust effect of prolonged sleep deprivation is a progressive increase in whole body energy expenditure, manifested by dramatic increases in food intake with loss of body weight [9,27]. Body temperature increases slightly by less than 0.5 °C above nor-

mal during the first half of the sleep deprivation period and then declines to basal levels three-fourths of the way through the deprivation period. During the last several days of survival, mild hypothermia of 1 °C below normal develops, which can worsen either abruptly or at varying rates over a few days [9,10]. The late development of hypothermia suggests an impairment of heat retention mechanisms because it occurs in spite of high rates of whole body energy expenditure [9,52].

After the first few days of sleep deprivation, small erythematous papules appear on the skin of the tail, mostly the dorsal and lateral aspects, and on the plantar surface of the paws [27]. These skin dermatoses develop into well-circumscribed hyperkeratotic, ulcerative and necrotic lesions [41]. Some possible causes of these skin problems, such as zinc deficiency, necrotizing vasculitis and mechanical variables, have been ruled out [31,41].

Until very late in the course of sleep deprivation, most clinical chemistry and hematological parameters are either within normal limits or show no sufficient disturbances which might indicate a dysfunction in an organ or system, e.g., renal or hepatic [27]. Still, two potentially serious conditions do evolve: anemia (normocytic), which would be expected to compound the increased oxygen demands of hypercatabolism, and low plasma albumin concentrations, which are suggestive of problems associated with limited amino acid availability [26].

The progressively deepening energy debt that characterizes most of the sleep-deprivation period eventuates in an acute cachectic-like phase. Core temperature declines to mild hypothermia (~ 1 °C below basal levels, which would not be expected to have an adverse effect if induced experimentally by other means in a normal animal). The energy debt worsens and body weight declines further to 12–20% below baseline [25,27]. Yet, hyperphagia often continues at near unprecedented rates [25]. Acute changes in clinical chemistry parameters are sometimes observed. These may include increased plasma glucose, urea, uric acid and lactate dehydrogenase [31]. There are no seizures, convulsions, diarrhea or similar types of morbidity that often accompany physiological imbalances in other clinical situations. The cachectic-like state progresses to a moribund state, which is characterized by an appearance of weakness, usually over many hours [27,52,60]. The amplitude of the EEG decreases [27,60], probably due to low brain temperature [52], and the subjects fail to generate many high voltage EEG waves. Despite impending death, morpho- and histopathological examinations have not suggested abnormalities in any major organ [27,32], but there is little white adipose tissue in the peritoneum and the connective tissues lack a normal fatty appearance [27]. Adrenal glands are enlarged when morbidity is advanced [27].

If a function of sleep is to conserve energy [2,8,84], then sleep deprivation might be expected to be an antithesis, a state of net catabolism. However, the energy expenditure seems to be far in excess of that required by normal waking activities and physiological explanations for this are not clear. Many likely explanations have been eliminated through extensive study; among them, changes in gross locomotor activity [9], diabetes [27], distress [59] and changes in total body water [9]. Malabsorption problems or blockage of pathways of intermediary metabolism of glucose, protein or fat have not been found [9,27], indicating that the sleep-deprived rats are able to utilize the energy that they have consumed.

Except for thyroid hormones, which decline in the plasma (discussed below) [9], changes in neuroendocrine markers have not indicated a dysfunction per se, but can be interpreted as responses to metabolic demands. For example, plasma norepinephrine and epinephrine show a progressive increase that appears to parallel that of the energy expenditure, with norepinephrine showing the more marked increase [9]. If norepinephrine is blocked by the post-ganglionic blocker, guanethidine, plasma epinephrine increases in a compensatory manner and the negative energy balance is maintained as before [55]. A late increase in corticosteroids is sometimes found [9], which seems to be reciprocal to a decline in body temperature to below baseline levels.

It is not yet possible to predict which rats, prior to a moribund state but after prolonged sleep deprivation, will survive subsequent recovery sleep. For survivors, recovery sleep is marked by a dramatic rebound of rapid eye movement (REM) sleep [28]. Unlike short-term sleep deprivation, non-rapid eye movement (NREM) sleep rebounds have not been observed although most of the lost sleep was of this type [28]. Reversal of sleep deprivation-induced changes (e.g., increased food consumption and catecholamine concentrations) occurs quickly, with dramatic changes evident within the first day [28]. After recovery, no permanent damage has been found and the quick reversal of symptomatology suggests that the underlying critical factors induced by sleep deprivation are not cell damage, neuronal degeneration or primary organ failure, which would be expected to take a long time to recover.

3. Perspectives on the utility of categorizing the response to sleep deprivation as a general-adaptation-syndrome

There have been suggestions that sleep deprivation-induced effects can be best understood by examining them in light of concepts embodied in Selye's General-Adaptation-Syndrome (GAS) [65]. According to Selye, a syndrome is composed of bodily changes that are attempts to react to, resist and adapt to overwhelming circumstances (e.g., malignancies, severe burns, exposure to extreme heat or cold). These bodily changes can, in themselves, cause adverse reactions and may be ultimately insufficient to maintain equilibrium ('ultimately maladaptive') and end in death. Selye proposes, thus, a single class of stimuli, i.e., derailments of homeostasis. By definition then, most all bodily reactions are 'non-specific' because they can be common to more than one 'disease of adaptation', irrespective of the root cause, and these coexist with a small number of changes that are necessarily 'specific'. In accordance with the notion of non-specificity, for example, is a conclusion that the adrenal hypertrophy in moribund sleep-deprived rats is a common somatic response to processes involved in impending death. There has been speculation that the GAS can explain sleep deprivation's effects, because sleep deprivation is a state that eventuates in mortality from obvious disruption of homeostatic processes. Also, there is a recruitment of energy reserves and bodily changes that are not, in themselves, unique (e.g., weight loss, skin dermatoses). Therefore, the matter of the GAS is briefly addressed below.

The appeal of the Selye theory and the historic contributions (1932–1950) notwithstanding, it is not necessary to conform to the language and concepts embodied in the GAS theory to describe sleep deprivation's effects and it is disadvantageous for several reasons. While there is general agreement that sleep deprivation disrupts homeostatic processes, the *utility* of GAS tenets is questionable and it has been a focus of controversy elsewhere for many years [47]. First, in Selye's GAS, a myriad of possible bodily reactions may accompany a state of disrupted homeostasis. There is, therefore, no power to predict which will or will not occur, or, why. Which of all possible abnormalities should sleep deprivation be expected to produce? Second, certain bodily reactions that are considered highly characteristic of the GAS, because they tend to occur in many different kinds of deranged homeostatic states, do not necessarily occur in sleep deprived rats. For example, one such reaction, hemorrhagic stomach ulcerations, occur in starved rats and distressed rats, but not in sleep-deprived rats. There is therefore little support for the usefulness of GAS as a working framework if some very core or exemplary bodily reactions of the GAS are not found. To persist in favor of the GAS as an overall unifying theme to describe pathophysiology thus requires either overlooking the fact that certain symptoms did not occur or the summoning of the necessary hypothetical elaborations from the theory (made possible by the theory's sweeping scope). Third, the use of Selye's tenets does not obviate the need to conduct further investigations to determine the mediation of physiological changes that are found. (As might be remembered, Selye's assignments of 'specific'

and 'non-specific' changes were determined in a limited number of conditions for which the pathophysiology was well delineated.) And lastly, there is a great risk that GAS terminology, such as 'non-specific', can easily be confused or misconstrued to suggest spuriousness or unimportance. It would be preferable to use other terminology to describe what is meant with greater clarity; e.g., 'side effect' or 'secondary effect'. For these reasons, the GAS theory is not necessary to recognize both important similarities and important differences among disrupted homeostatic states. The fact that sleep deprivation results in a distinctive clinicopathological profile, which does not otherwise occur without sleep deprivation, suggests that the symptoms that comprise the profile are a highly specific set of responses to a missing requirement(s).

4. Nutritional and metabolic adaptations to prolonged sleep deprivation

Two features of the sleep deprivation profile emerged as the principal consequences of sustained sleep loss. The first feature is the progressive increase in whole body energy expenditure [27]. The second feature is a collection of symptoms that resemble malnutrition [31]: hypoalbuminemia (the hallmark of protein deficiency), anemia, low plasma thyroxine concentration, weight loss, a wasted appearance with poor fur condition, skin dermatoses and a negative energy balance. (Malnutrition is distinguished from starvation, in which metabolism is lowered in compensation for limited availability of all nutrients.) These two features could be causally related to one another. On the one hand, the hypercatabolism and negative energy balance produced by sleep deprivation could result in malnutrition and anabolic deficiencies. On the other hand, if sleep promotes biosynthetic activities and tissue restoration, as some researchers have proposed [54], energy expenditure during sleep deprivation could rise as a consequence of increased metabolic work, due to biosynthetic activities that might be both difficult without sleep and increased to balance any escalation in protein degradation.

Rats are normally exquisite regulators of body weight. If protein demands are high, e.g., during pregnancy or dietary deficiency, rats will increase their intake of food to meet their protein requirements [16,49] and energy expenditure rises as a consequence of disposal of non-protein calories through non-shivering thermogenesis [62,79]. Under most other conditions, rats regulate body weight by adjusting caloric intake [34]. To elucidate possible causal relationships between protein and energy deficits during sleep deprivation, rats were fed balanced diets that were augmented with either protein or calories to determine

which, if any, symptoms of sleep deprivation might be alleviated or were intractable [31].

During normal baseline conditions, body weight regulation was minimally influenced by the type of diet provided. The protein-augmented diet was isocaloric to a normal laboratory diet and was comprised of 40% protein, rather than 25%. The fat and calorie-augmented diet had the same amount of protein as a normal laboratory diet and the caloric density was increased 45% above normal by the addition of oils and milk solids. Both diets were nutritionally balanced and the dilution of other essential nutrients, such as vitamins and minerals, was avoided. Rats fed the high fat and calorie (HC) diet consumed 17% fewer grams of food and regulated body weight slightly above (7%) that of rats fed the high protein (HP) diet. The somewhat greater food intake in the HP diet rats indicated that the HP diet did not suppress appetite.

Under the conditions of sleep deprivation, diet composition profoundly altered food intake and body weight. Sleep-deprived rats fed the HC diet showed progressive increases in food consumption of 200–300% of normal amounts without a gain in body weight (Fig. 1). Sleep-deprived rats fed the HP diet consumed a normal amount of calories throughout the experimental period; however, the development of skin dermatoses was hastened and body weight loss was greater than 16%. Survival ability (i.e., the number of days of sleep deprivation required to produce a decline in core temperature to $> 1\,°C$ below basal levels along with a feeble appearance) was 40%
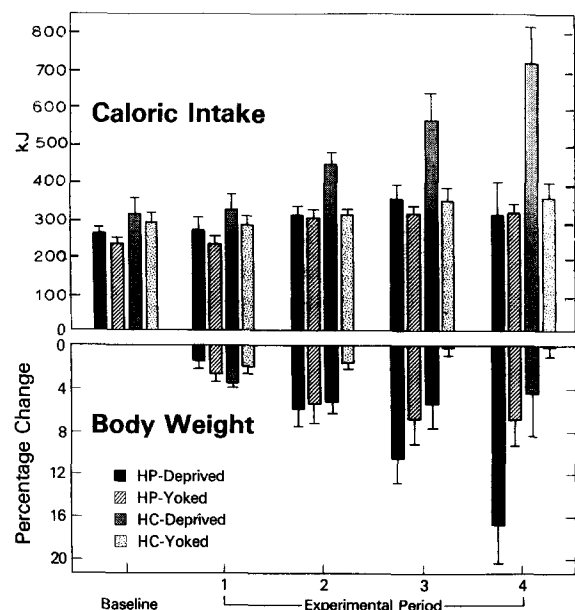

Fig. 1. Caloric intake (in Joules) (top) and body weight (bottom) across baseline and four consecutive intervals of 25% of the experimental period in sleep-deprived and yoked-control groups fed diets augmented with protein (HP) or calories (HC). Error bars, SE. Adapted from and reprinted with permission from Ref. 31.

longer in rats fed the HC diet than those fed the HP diet (an average of 26 vs. 18 days, respectively). Comparison yoked-control groups did not appreciably alter their intake of either diet and remained healthy, pointing to a marked interaction between sleep deprivation and diet composition in the sleep-deprived groups (Fig. 1).

The adaptive and survival benefits of the HC diet is attributable not only to its caloric density (38 kJ/g of metabolizable energy for fat compared with 17 kJ/g for protein or carbohydrate), which allows more food to be consumed before the capacity of the digestive system to process food bulk is reached [34], but also to the marked hyperphagia induced by sleep deprivation. Metabolic demands raise the requirements for all nutrients. Not only did the sleep-deprived rats fed the HC diet consume more calories, but they consumed an amount of protein that was comparable to that consumed by sleep-deprived rats fed the HP diet. Therefore, to meet the increased metabolic demands of sleep deprivation probably requires a balanced diet; increased caloric intake without additional nutritional benefit would have created a dietary imbalance, which tends to produce aphagia [34].

In the face of the increased energy requirements of sleep deprivation, the HP-fed sleep-deprived rats seemed to exhibit adaptive behavior by losing weight instead of by eating more. Protein has a much lower efficiency of utilization than does fat or carbohydrates and metabolic expenses are raised with the act of food consumption and protein breakdown [15]. For this group, then, an increase in caloric intake in the form of protein would probably be of little benefit, whereas weight loss was probably a less costly and more adaptive mode by which to furnish energy substrates.

Some of the clinical manifestations of sleep deprivation (e.g., weight loss, hypoalbuminemia, anemia) are, therefore, not intractable, but appear to be manifested to a greater or lesser extent depending on the type of dietary nutrients and the energy available and the underlying metabolic response (e.g., gluconeogenesis, ketogenesis). In malnutrition, the clinical manifestations differ along a continuum from calorie undernutrition (marasmus) to protein-caloric malnutrition to protein malnutrition with adequate calories (i.e., Kwashiorkor) and reflect the changes in underlying enzymatic and cellular processes in response to the type of nutritional deficit [76]. By way of illustration, hypoalbuminemia can be induced experimentally in rats by feeding a diet relatively high in energy but below maintenance in protein [44,45]. In a similar manner, despite the survival disadvantage of the HP diet, some sleep deprivation-induced symptoms, e.g., hypoalbuminemia and anemia, were less severe in the sleep-deprived rats fed the HP diet than in hyperphagic, sleep-deprived rats fed a normal diet [26]. Thus, diet composition inter-

acts strongly enough with sleep deprivation to alter the severity of some symptoms and to vary survival ability.

Sleep deprivation resembles other disorders characterized by primary hypercatabolism. In sleep-deprived rats, there are several indications that nutrient turnover is increased: glucose clearance from the plasma is accelerated [27] and levels of plasma cholesterol, triglycerides or glucose do not become abnormal despite intake of fat and calories that is 200–300% above normal [31]. Similarly, in hyperthyroidism, for example, the changes in intermediary metabolism include: (1) increased glucose absorption from the intestine and increased glucose utilization and removal from the plasma, although plasma concentrations remain normal; (2) accelerated protein formation and degradation; and (3) increased lipid mobilization, synthesis and degradation [43]. Provided that protein intake is adequate, caloric augmentation alleviates the effects of protein degradation better than protein augmentation [24]. In cancer, as a second example, glucose turnover is increased in both the fasted and the fed state, oxidation of lipids continues despite glucose infusion, and protein turnover rates are increased, all indicating a failure to adapt to diminished energy supplies [48]. In cancer patients, metabolic complications are reduced, in part, by the addition of fat to glucose-based total parenteral nutrition, which limits the metabolic cost of converting glucose to fat [48]. Then, by extension, regardless of the root of the negative energy balance in sleep-deprived rats, the beneficial effects of fat and calorie augmentation would be expected to exceed that of protein augmentation alone (even if there are coexistent problems with protein biosynthetic processes).

Diet composition is the most potent variable known to alter to the time course of sleep deprivation and the development of symptoms in these sleep-deprived rats. However, the negative energy balance per se cannot explain why the sleep-deprived state becomes life-threatening. This is illustrated by the fact that the HC-fed sleep-deprived group maintained their body weight and some white adipose tissue and yet the critical outcomes were not postponed indefinitely.

5. Thyroid hormones

Despite an outward resemblance of sleep deprivation to hyperthyroidism (i.e., increased energy expenditure, weight loss and hyperphagia), the plasma total thyroxine (TT_4) concentration in sleep-deprived rats declines progressively, reaching a level below assay detection in some cases [9]. The average TT_4 decline during the last several days of the experimental period is 79% below baseline. Plasma total triiodothyronine (TT_3) also declines, but not as severely, to 52% below baseline [9]. The cause of the decline

in TT_4 is unknown: it cannot be explained by increased conversion and disposal, since other comparable states of increased metabolic demands, such as hyperthyroidism [21] and long-term cold exposure [4,68], are not associated with difficulties in maintaining or increasing plasma levels of T_4 and T_3, despite increased T_4 clearance. In present studies, the pituitary-thyroid axis is challenged by administration of the hypothalamic hormone, thyrotropin-releasing hormone (TRH). The pituitary release of thyrotropin (TSH) both before and after TRH administration and the thyroidal release of free (i.e., unbound and biologically active fractions) T_4 and T_3 in response to the TSH rise will help to clarify whether there is a dysfunction of the pituitary or thyroid gland or an alteration in a central regulatory mechanism that might be responsible for the low T_4 (C.A. Everson and H.L. Reed, unpublished study). For example, low plasma T_4 is a potent stimulator of TSH release [80], which, in turn, stimulates the thyroid gland. TSH would be expected to be increased in sleep-deprived rats if the hypothalamic and the pituitary regulatory mechanisms are functioning normally.

In the periphery, the low plasma TT_4 and TT_3 concentrations appear to be an integral part of sleep-deprivation symptomatology, including the deep negative energy balance. As plasma total thyroid hormones decline, catecholamines rise [9,56]. In a variety of other situations, besides sleep-deprivation, there is a striking reciprocal relationship between plasma thyroid hormone decreases and catecholamine increases [63,67]. For example, in some metabolically complicated states, such as severe burn injury, profound hypermetabolism may be mediated, in part, by high plasma catecholamine levels that are reciprocal to low T_3 [6]. In experimental thyroid hormone deficiency in rats, catecholamine synthesis and release is accelerated, which results in an enhanced thermogenesis [63] and a marked increase in local T_4 to T_3 conversion by $5'$-deiodinase type II ($5'$D-II) in brown adipose tissue (BAT) [67,69]. The $5'$D-II pathway has been estimated to contribute as much as 40% of the circulating T_3 [69].

In sleep-deprived rats, the activity of BAT $5'$D-II is increased 100-fold [5]. Administration of thyroxine fails to completely suppress this increase as expected [5], which indicates that other factors, in addition to low plasma T_4, are contributing to BAT activation. One such factor might be glucagon, which is known to be highly catabolic and a stimulant of BAT $5'$D-II activity [70] and mitochondrial nucleotide binding [12], and which is increased in sleep-deprived rats (C.A. Everson, unpublished findings). Another factor might be the increase in calorie and carbohydrate consumption that these animals exhibit, which is associated with increased T_3 production in euthyroid rats, presumably by the same mechanisms of catecholamine release and increased BAT $5'$D-II activity [20] present in

hypothyroid rats. Thus, during sleep deprivation, changes in peripheral hormones that have the capacity to be highly catabolic might be both compensatory responses to low thyroxine availability and mediators of the negative energy balance. Furthermore, low thyroid hormone concentrations may contribute to other sleep deprivation-induced pathologies, such as the eventual lowering of core body temperature and the development of skin dermatoses.

6. Brain function during prolonged sleep deprivation

Despite the marked peripheral effects of sleep deprivation in rats, gross perturbations in the chronically awake brain have not been found. Histopathological examination of sleep-deprived rat brains, even when death was imminent, revealed no structural damage, cell death or neuronal degeneration different from background variation of their yoked-control partners [32]. Nor have neuromodulatory abnormalities been found, as measured by monoaminergic concentrations and turnover [11]. More reductionistic inquiries into the regulation of the central noradrenergic system (i.e., α_1-, α_2- and β-adrenergic) failed to find a group difference in receptor number and affinity [78]. A search for a change in early gene expression, i.e., Egr-1-like immunoreactivity, also failed to find differences from yoked controls [42]. While one could argue that this abundance of negative findings on structural and biochemical changes could indicate that sleep deprivation does not much affect the brain, it is more likely the case that effects are not likely to be generalized throughout the brain.

The deep negative energy balance that develops in the sleep-deprived rats and the cognitive deficiencies found in humans, which are readily reversible with sleep [36], suggested a functional, metabolic disturbance during prolonged wakefulness. Increased functional activity in the nervous system is generally accompanied by increased glucose utilization in the activated regions. To assess and map functional activity in individual structures of sleep-deprived and yoked-control brains, we therefore measured local rates of cerebral glucose utilization by the quantitative autoradiographic 2-[^{14}C]deoxyglucose (2[^{14}C]DG) method [29].

Rats were fed ad libitum the fat- and calorie-augmented diet [31] discussed above to help meet the increased energy requirements expected during sleep deprivation to avoid possible confounding effects of starvation-like responses by the brain. Sleep deprivation was continued for 11–12 days, a period long enough to raise whole body energy metabolism and thus increase the likelihood that factors were operating that might be reflected in measurable changes in the brain, but short enough to preclude the effects of debilitation that develop later. At the time of the

2[^{14}C]DG procedure, all the usual conditions of the sleep-deprivation paradigm were in effect and the sleep-deprived rats and the yoked-control comparison rats were awake, unanesthetized and freely moving. Waking brain and core temperatures were recorded in a separate group of sleep-deprived and yoked-control rats that were treated similarly to those in which brain metabolism was measured. By the 11th experimental day, waking brain temperature had increased from baseline by 0.4 °C in sleep-deprived rats, but not in yoked controls. This increase in waking brain temperature was not due to warm peripheral blood because core temperature remained at a basal level.

Local rates of cerebral glucose utilization were not elevated by sleep deprivation in any of the 60 structures examined, despite systemic hypermetabolism, increased deep brain temperature and sympathetic activation, all of which would be expected to be reflected by increased brain activity. Average glucose utilization in the brain as a whole was unchanged: 76 ± 3 (S.E.M.) vs. 70 ± 2 (S.E.M.) μmol/ 100 g/min in yoked-control and sleep-deprived groups, respectively. However, regional decreases were found. The most marked decreases were concentrated within the hypothalamus, thalamus and limbic system. Mesencephalic and pontine regions were relatively unaffected except for the central grey area. The medulla was entirely normal. The effects of sleep deprivation on the brain tended, therefore, to be unidirectional and heterogenous among structures and dissociated from the energy metabolism of the periphery. The alterations in metabolic activity were moderate rather than severe (i.e., significant paired differences from the yoked controls were reductions of 7–20%), but they were determined midway through the sleep-deprivation period at a time when other symptomatology was also moderate. Since most major changes induced by sleep deprivation are progressive in nature, the reductions in cerebral metabolism also would be expected to become more severe with time. Illustrations of several anatomical regions and their corresponding activation levels are shown in eight representative autoradiograms in Fig. 2.

Utilization of ketone bodies by the brain as alternative energy substrates was a concern because the brain has been shown to use ketone bodies more or less in proportion to their concentration in the blood [61]. Therefore, the concentration of D-β-hydroxybutyrate (β-OHB) was determined in plasma samples collected throughout the course of the sleep deprivation period. The enhanced diet induced a mild degree of ketosis in both sleep-deprived and yoked-control groups, but the plasma concentration of β-OHB was not significantly different between groups, nor was it sufficient to depress glucose utilization below that found in unanesthetized rats of approximately the same age (69–77 μmol/100 g/min [71,72]).

The rat normally sleeps 50% of the time and cerebral

metabolism is reduced by 30% [37]. As such, the sleep-deprived rat would not have received any putative benefit of a sleep-related lowering of cerebral metabolic rate. Decreases in cerebral metabolism observed during sleep deprivation might suggest a compensatory action to conserve energy or to maintain a sleep-like state. This is unlikely, however, because the average rate of glucose utilization in the brain as a whole did not differ significantly between yoked-control and sleep-deprived rats, whereas the sleep-related decrease in cerebral metabolism is generalized throughout the brain. The structures that were affected in the sleep-deprived brain were heterogeneously distributed. Furthermore, the metabolic activities of some of the largest and most metabolically active areas, which might be expected to be the most affected, remained normal. These structures included the mammillary body, inferior colliculus and several cortical regions. In contrast, some of the brain regions that normally have a low metabolic activity (and, therefore, low cost) failed to remain normal.

There have been suggestions that the increased total body metabolism, increased brain temperature and sympathetic activation that these animals exhibit can be explained by behavioral factors, such as hyperarousal, distress and hypervigilance. However, these states are associated with increased cerebral metabolism, not decreased, as found in sleep-deprived rats. In humans, 'apprehension' is associated with a general increase in brain metabolism of 36% [38]. In rats, stress produced by tubocurarine paralysis, causes increases of 34–145%, depending on the region measured [17]. Increases in local rates of cerebral metabolism are also found in states associated with peripheral features of hyperactivity and restlessness, induced in rats by amphetamine administration [53]. Therefore, the unidirectional effects toward decreased brain metabolism do not support speculations that peripheral effects of sleep deprivation are due to emotional arousal.

The hypometabolic brain regions in sleep-deprived rats do, however, suggest a correspondence between the functions served by these regions and the peripheral symptomatology. In the hypothalamus, for example, there are foci for thermoregulation, endocrine regulation, host defense responses and sleep. Experimental lesions of the ventromedial hypothalamus in rats produce profound hyperphagia [33,64], which appears to be paralleled only by the hyperphagia of sleep-deprived rats. Conversely, the lateral hypothalamus, a region known to cause aphagia and lowered body weight setpoint when lesioned, remained unchanged in sleep-deprived rats. With regard to thyroid hormone regulation, discussed above, several hypothalamic regions that become hypometabolic are also known to be near foci of TRH regulation [1,46], which raises the likelihood that central mechanisms are involved in alter-
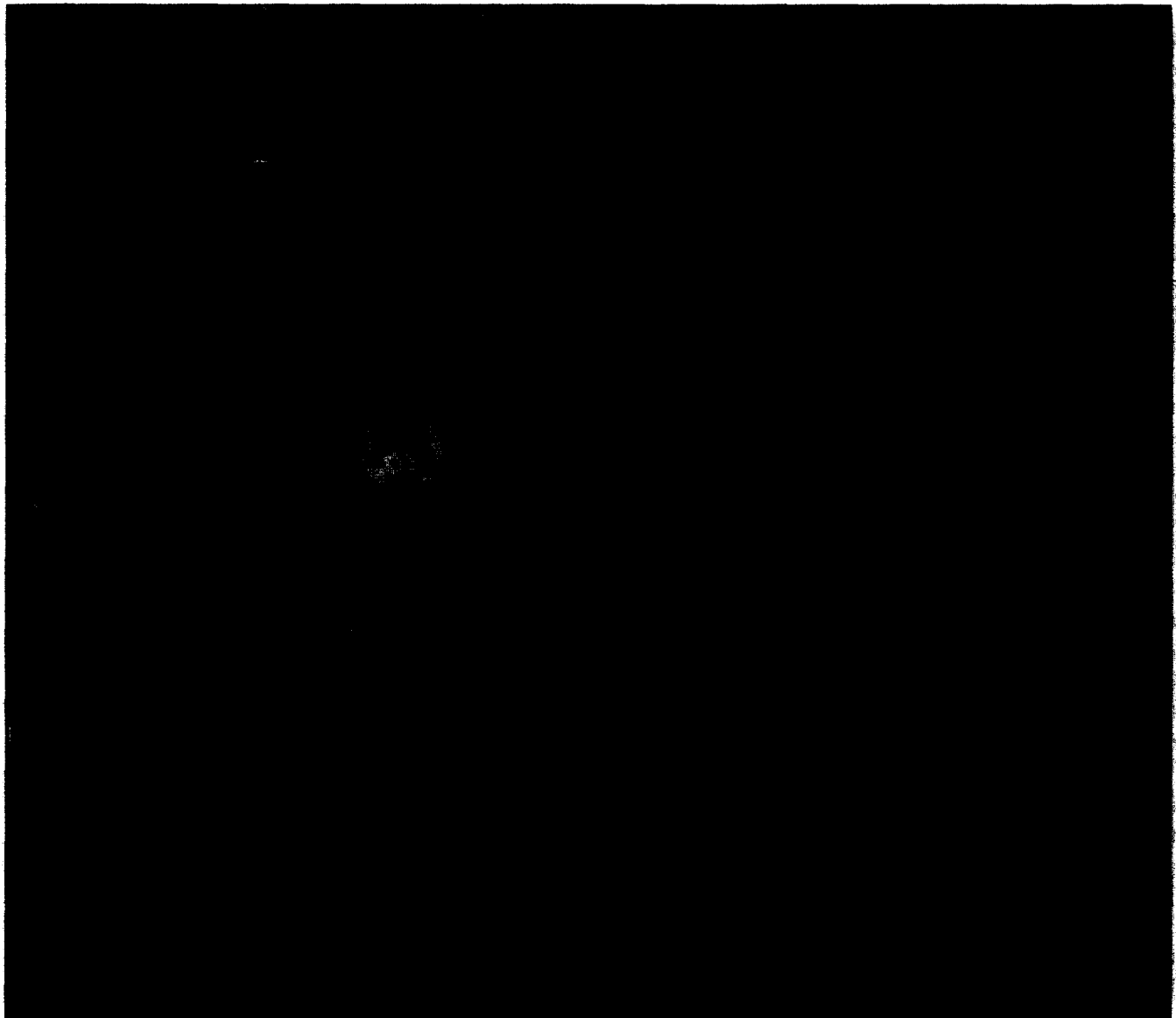
Fig. 2. Computer-generated quantitative color-coded autoradiograms of coronal sections at four different levels in the brains of rats representative of yoked-control and sleep-deprived groups. The color bar on the right provides the calibration scale for the range of values of rates of glucose utilization in μmol/100 g/min for each color. The scale bar on the lower right represents 2.5 mm. Abbreviations are as follows: LS, lateral septal nucleus; GP, globus pallidus; AH, anterior hypothalamus; MG, medial geniculate body; SNR, substantia nigra, reticulata; SC, superior colliculus; DR, dorsal raphe nucleus; PMR, paramedian raphe nucleus; Gi, gigantocellular reticular nucleus. Of the labeled structures, paired statistical differences, sleep deprived vs. yoked control, were significant at $P<0.05$ for LS and $P<0.02$ for AH. Adapted from Ref. 29.

ations in thyroid hormone regulation in the sleep-deprived rat. In addition, the sleep-deprived rat develops hypothermia despite increased whole body energy expenditure [9] and also exhibits heat seeking behavior [57]. In a similar manner, experimental lesions of the preoptic regions of the hypothalamus results in impaired autonomic, but not behavioral, thermoregulatory reflexes resulting in an inability to defend body temperature [74,75]. The hypothalamus is also known to have an important role in fever production and host defense responses [14,35,66] and, as discussed below, the ability of sleep-deprived rats to mount appropriate inflammatory responses is questionable.

7. Host defense

The reason that sleep-deprived rats eventually succumb to sleep loss and die has eluded explanation for many years. Recent evidence has shown that the lethal factor is a decreased resistance to infection. Host defense breaks down and lethal opportunistic organisms that the body normally controls enter the bloodstream and presumably trigger a cascade of toxic reactions that lead to death [25]. Examination of blood cultures from sleep-deprived rats to look for pathogenic organisms (blood poisoning) was prompted by the similarities between the eventual physical condition of the sleep-deprived rat and several features

of toxic states; e.g., there is a lack of structural damage, possibly due to pathogenesis in a diffuse system; a lack of evidence that any organ-system is dysfunctional; quickly reversible changes (with sleep), suggesting that severe permanent damage had not occurred; and the development of a cachectic-like state. Furthermore, opportunistic organisms were suspected because morphological changes that might induce toxic-like factors and reactions, such as tumors in cancer patients, are conspicuously absent in the sleep-deprived rat. Toxic states also lack strong prognostic indicators and yet are highly lethal [18]; a situation that could explain why it is difficult to determine which sleep-deprived rats will live when permitted to sleep once again.

In the study [25], the sleep-deprivation period was continued until the life of the rat appeared to be threatened, as evidenced by a drop in body temperature to >1 °C below baseline (Fig. 3), but not to the point of physical incapacitation. [Sleep-deprived rats were motorically active, oriented and had consumed food within the previous 24 h (Table 1)]. Lethal opportunistic microbes were indeed cultured from heart blood from five of six sleep-ueprived rats, but not from their yoked-control partners or any surgery-control rat (Table 1). The significance of this finding is accentuated by the fact that the normal rat is well-known, if not notorious, for its resistance to infection. Because the symptoms of sleep deprivation are near-identical among rats, the sixth sleep-deprived rat might have had systemic infection that was not detected because of limitations on successful recovery rate that are inherent to blood cultures [3].

There appeared to be little physiological opposition to the systemic infection. Febrile spikes were not observed at any time during the experimental period (monitored every

Table 1
Status of individual sleep-deprived rats the day before and the day of examination

Rat #	Day	24-h change from baseline			Microbes cultured from blood
		Food (%)	Weight (%)	Temp. (°C)	
1	20	77	− 8	− 0.9	
	21	130	− 8	− 1.1	Streptococcus agalactiae
2	22	125	− 24	− 0.5	
	23	55	− 28	− 1.7	Pseudomonas aeruginosa
3	21	103	− 26	− 0.6	Corynebacterium, Grp JK,
	22	91	− 30	− 1.5	Staph. & Bacillus Sp.
4	26	46	− 25		Pseudomonas aeruginosa,
	27	− 35	− 27	ND*	Staph. aureus
5	15	151	− 15	− 0.3	
	16	238	− 16	− 1.5	none
6	20	84	− 14	0.1	
	21	− 56	− 20	− 1.1	Pseudomonas aeruginosa

* ND, not determined due to transmitter failure.
Adapted with permission from ref. 25.

30 s) and body temperature fluctuations did not exceed an expected maximal level. Also, histopathological evaluation revealed that the tissue responses were poor compared with those typically found in most infectious disease states.

With the knowledge that sleep deprivation eventuates in bacteremia and septicemia, several other sleep deprivation-induced effects besides mortality can be viewed from a new perspective. For example, bacteremia provides a likely explanation for the drop in core temperature while energy expenditure is high. Hypothermia associated with
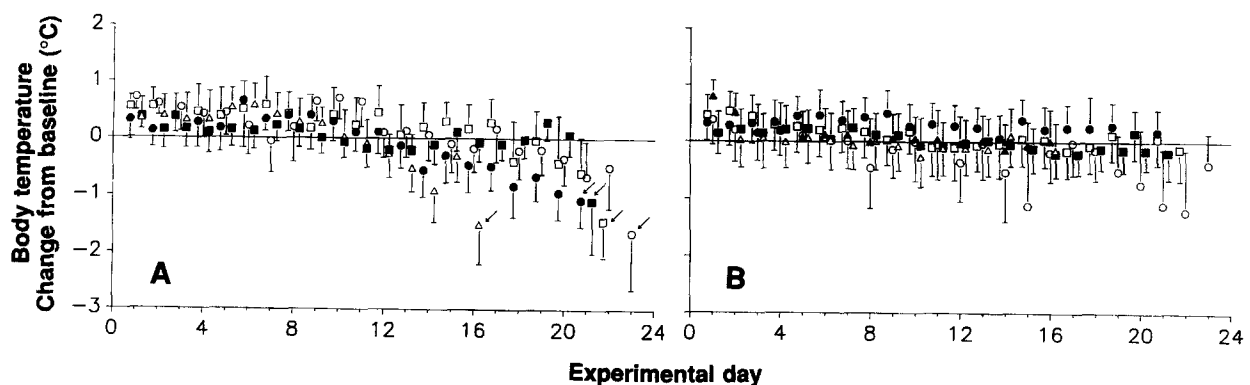

Fig. 3. Mean daily change (± S.D.) of body temperature from the baseline average per subject (● = Experiment (EXP) 1, ○ = EXP 2, □ = EXP 3, ▲ = EXP 4 (temperature recordings in the sleep-deprived rat were lost due to transmitter failure), △ = EXP 5, ■ = EXP 6) across experimental days in sleep-deprived (A) and yoked-control (B) rats, calculated from 2860 epochs of 30-s temperature averages per day. Arrows (✓) indicate the day the core temperature of sleep-deprived rats had declined >1 °C of baseline and heart blood was taken for microbial cultures. The grand, normal baseline temperature range was ±0.5 °C SD of the mean and included both the temperature minima associated with sleep as well as the maxima associated with wakefulness. Accordingly, minor elevations above the baseline mean partially reflect a greater percentage of time spent in wakefulness. No fever was detected in sleep-deprived rats in spite of systemic infection. These data indicate that daily body temperature is regulated within close limits of the daily mean and that the daily means of sleep-deprived rats decline below baseline until hypothermia becomes profound. Reprinted with permission from Ref. 25.

bacteremia is different from hypothermia due to drugs or climatic exposure and is characterized by a high cardiac index and low vascular resistance [51]. Therefore, the decline in body temperature in sleep-deprived rats to below normal levels (Fig. 3) indicative of pathological heat loss, might be attributable to impaired vasoconstriction secondary to a host defense breakdown. Also, lowering of body temperature, whether secondary to bacteremia or other impaired mechanisms, might preclude fever, which has been shown to have survival value during infection [39]. Sleep-deprived rats also develop skin lesions that are necrotic and do not become inflamed [41] and share features with dermatoses present in immunocompromised states, such as the ecthyma gangrenosa of *Pseudomonas aeruginosa* infection [81]. Cutaneous manifestations are often first overt signs of a primary alteration in the general susceptibility of the host to pathogens and reflect systemic disease, as well as being potential portals of entry for pathogens [81]. The lesions themselves would not be expected to cause lethal bacteremia without other pre-existing host impairments; e.g., lack of inflammatory reactions. Thus, several sleep deprivation-induced effects indicate that host defense may be compromised in a variety of ways.

The results indicate that sustained wakefulness resulted in decreased resistance to infection in a once-normal animal. These findings, therefore, strongly support the proposition that sleep has a vital role in immune function. Ensuing questions, among others, are, how early is host defense a problem for sleep-deprived rats? Does sleep deprivation affect immune function directly? Systemic infection is, and would be expected to be, a late condition. Also, bacteria have not been found in the blood of sleep-deprived rats prior to a life-threatened state (C.A. Everson, unpublished observations). However, several predisposing events must be developing over days and perhaps weeks, to culminate in a state wherein bacteria are permitted to infect the bloodstream. The lack of a febrile response during systemic infection, along with poor tissue inflammatory reactions and necrotic skin lesions, are suggestive of earlier immunosuppression. Compromised integrity of the host defense system would be expected to induce strong responses by immune function mechanisms, such as cytokines and other immunomodulators. Cytokines are known for their highly catabolic effects. Furthermore, bacterial pathogens need not be present for cytokines to become overactivated and cause adverse physiological reactions [77,83]. Thus, on the one hand, the syndromic course of sleep deprivation shares several features with what could be expected during a chronic septic challenge [19,22,58,73,77,81,82]: (1) progressive development of a deep negative energy balance despite normally sufficient food intake [27,31], conceivably mediated in part

by catabolic immunomodulators; (2) progressive elevations in plasma norepinephrine [9], alkaline phosphatase [30] and blood leukocytes [31]; (3) increased heart rate [27]; (4) early skin dermatoses that necrose but do not become inflamed [41]; (5) suppressed plasma thyroxine and triiodothyronine [9]; and (6) a late, mild increase in corticosteroids that mirror declining body temperature [9].

On the other hand, decreased resistance to infection might not be primary to sleep deprivation but secondary to other circumstances induced by sleep deprivation: the high energy expenditure, which might rob energy from vital processes; malnutrition, for which infection is always a high risk; loss of body temperature and associated decreases in total thyroid hormone concentrations; and decreased functional activity of brain regions that are foci for host defense reactions.

8. Conclusion

The nature of sleep deprivation's effects include: (1) increased whole body metabolism and associated malnutrition-like symptoms; (2) heterogeneous and unidirectional effects on cerebral structures toward decreased function; (3) low concentrations of plasma thyroid hormones; and (4) decreased resistance to infection. Because of strong interrelationships, each effect can be interpreted as the primary effect that could result in the others. For example, host defense was the first system found to fail, but mild regional decreases in brain function found after a shorter duration of sleep deprivation could conceivably begin the process of altered homeostasis and induction of counterregulatory mechanisms. Therefore, the findings remain somewhat equivocal for a unitary function for sleep, but can support several putative roles for sleep in thermoregulation, energy conservation, immune system integrity and tissue restoration.

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