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Effects of acute and chronic sleep loss on immune modulation of rats

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Zager A, Andersen ML, Ruiz FS, Antunes IB, Tufik S. Effects of acute and chronic sleep loss on immune modulation of rats. *Am J Physiol Regul Integr Comp Physiol* 293: R504–R509, 2007. First published April 4, 2007; doi:10.1152/ajpregu.00105.2007.—Sleep deprivation is now recognized as an increasingly common condition inherent to modern society, and one that in many ways, is detrimental to certain physiological systems, namely, immune function. Although sleep is now viewed by a significant body of researchers as being essential for the proper working of a host of defense systems, the consequences of a lack of sleep on immune function remains to be fully comprehended. The aim of the current study was to investigate how paradoxical sleep deprivation (PSD) for 24 and 96 h and sleep restriction (SR) for 21 days by the modified multiple-platform method, and their respective 24-h recovery periods, affect immune activation in rats. To this end, we assessed circulating white blood cell counts, lymphocyte count within immune organs, as well as Ig and complement production. The data revealed that PSD for 96 h increased complement C3 and corticosterone concentration in relation to the control group. In contrast, the spleen weight, total leukocytes, and lymphocytes decreased during SR for 21 days when compared with the control group, although production of a certain class of immunoglobulin, the IgM, did increase. After recovery sleep, lymphocyte count in axillary lymph nodes grew when rats had rebound sleep after PSD for 24 h, neutrophils increased after PSD 96 h and lymphocytes numbers were higher after SR 21 days. Such alterations during sleep deprivation suggest only minor alterations of nonspecific immune parameters during acute PSD, and a significant impairment in cellular response during chronic SR.

sleep deprivation; immune system; host defenses; leukocytes; immunoglobulin

THE NUMBER OF PEOPLE WHO EXPERIENCE sleep deprivation (SD) is on the rise in societies that have a hectic lifestyle. The concept that modern society is chronically sleep deprived has found acceptance as of late, and the repercussions of sleep loss across broad physiological and systemic mechanisms are aspects that have lured the attention of a significant body of researchers. Despite the growing amount of literature on the topic, much remains to be comprehended about the physiological chain of events that underlie SD (23).

Only recently has the view that sleep is vital in various defense systems become ubiquitous among investigators. In spite of some controversy, it is now widely recognized that sleep loss leads to impairment of those defenses, thus rendering an organism more susceptible to pathogens (21, 22). SD expresses comorbidity with disease states in which deregulation of immune variables is considered a principal etiological or contributory factor (27, 33). Otherwise, during infection, animals that have robust sleep responses have a better prognosis for survival than those that do not (34).

During normal sleep, there is a redistribution of circulating lymphocyte subsets and an increase in some aspects of cellular immunity (4). In contrast, in clinical populations that have disordered sleep due to stress or a variety of diseases, decrements in natural and cellular immune function coincide with disturbances of detectable sleep architecture and loss of sleep (7, 8, 18, 19). Despite the intuitive understanding that sleep is critical for health and life, the mechanisms through which SD leads to impairment of the immune system are poorly elucidated. One of the most compelling findings associating SD to a breach in the immune system was reported in a study by Everson (15), in which an increased rate of bacteremia in rats chronically deprived of sleep was observed. Bacteria in blood cultures were found in five of the six rats sleep deprived to near-terminal conditions (15). Also observed were decreases in lymphocyte counts and natural killer (NK) cells during SD (5, 16, 28, 31), although in another study, SD led to increased numbers of lymphocytes and NK cells (4). Furthermore, an increase in antibody titers was found after SD in humans (17), rats (14), and mice (29, 30).

Understanding the effects of sleep and SD on the immune response could shed some light on how the brain affects immune function. The central feature of sleep is an alteration in brain function, which is associated with both direct and indirect changes in other physiological systems (6). In the current lifestyle, most of the deprivation of sleep occurs in the paradoxical sleep/rapid eye movement (PS/REM) phase, which takes place in the second half of the night. For that reason, many investigators have developed and used different approaches to reduce or abolish PS; therefore, most investigations of SD focus on paradoxical sleep deprivation (PSD). Yet another strategy used to mimic the sleep loss that society is subjected to is sleep restriction (SR), in which animals are submitted to gradual loss of sleep during long-term periods, also representing an important model of sleep consolidation and adaptation. When allowed to sleep, in both methodologies, animals compensated for the loss of sleep by spending more time in PS, which is a consequence of homeostatic sleep regulation and appears to be proportional to the amount of sleep lost during the deprivation period (24, 25).

Thus, the aim of this investigation was to examine how short-term PSD and long-term SR, and their recovery periods, affect immune activation by assessing circulating white blood cells, lymphocytes within the immune organs, and immunoglobulin and complement production.

MATERIALS AND METHODS

Animals

Ninety-day-old male Wistar-Hannover rats were bred and raised in the animal facility of the Centro de Desenvolvimento de Modelos

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Experimentais para Medicina e Biologia. The animals were housed in a colony maintained at 22°C with 12:12-h light-dark cycle (lights on at 0700) and allowed free access to food and water inside standard polypropylene cages. Rats used in this study were maintained and treated in accordance with the guidelines established by the Ethical and Practical Principles of the Use of Laboratory Animals (2).

PSD

The procedure consisted of placing 10 rats in a tiled water tank (123 × 44 × 44 cm) for 24 or 96 h. The tank contained 14 platforms (6.5 cm in diameters) rising 1 cm above water surface, thus allowing the rats to move around by leaping from one platform to another. At the onset of each PS episode, the animal experiences a loss of muscle tonus and falls into water, thus being awakened. The multiple platform procedure is effective in producing a total suppression of PS. A significant slow-wave sleep (SWS) loss also occurred throughout the 4 days of SD (−31%) (24). It therefore seems appropriate to consider these animals as being PS-deprived rather than being exclusively deprived of sleep. The cage control group was maintained in the same room the experimental rats were in for the duration of the study and showed normal sleep patterns, PS, SWS, and wake (24). Throughout the study, the experimental room was maintained under controlled temperature and a light-dark cycle. Food and water were available ad libitum, with chow pellets and water bottles provided on a grid located on top of the tank. The water in the tank was changed daily throughout the PSD period.

Sleep Restriction

The protocol consisted of submitting the rats to the modified multiple platform method, as described above for 18 h (beginning at 1600) for 21 days (SR period). After each 18-h sleep deprivation period, the rats were allowed to sleep for 6 h (sleep window beginning at 1000). Throughout the SR procedure, rats slept an average of 30–40% of the time, corresponding to 8–9 h per day. This time interval (1000 to 1600) was chosen because this is when PS attains its highest expression and when SWS homeostatic pressure is already yielded (25).

Experimental Procedure

Rats were randomly distributed into four groups: PSD, SR, recovery, and home-cage groups. Seven groups were obtained at the end of the experiment. The PSD groups PSD 24h and PSD 96h were subjected to PSD for 24 h (n = 10) and 96 h (n = 11), respectively. The SR group SR 21d was subjected to SR for 21 days. The recovery groups were sleep deprived or sleep restricted and returned to home cages and were allowed undisturbed, spontaneous sleep for 24h; corresponding to PSD24R (n = 10), PSD96R (n = 10) and SR21R (n = 10) groups. Finally, the control group (n = 10) was maintained in separate cages in the same room the experimental rats were housed during PSD, SR, and recovery procedures and were killed on the same day the other groups were. By housing all groups in the same room, we held control of those conditions for all groups. After residing in the water tanks (PSD and SR groups) or home cages (control and rebounds group), rats were brought to an adjacent room and decapitated between 0900 and 1200 with minimum discomfort.

Blood Sampling

Blood was collected into sterile tubes containing either liquid EDTA (differential leukocyte count and plasma corticosterone) or serum separator medium (immunoglobulin and complement concentrations). Differential leukocyte counts were determined in blood on an automatic blood cell counter (Advia 120 Hematology System, NY), on the same day that the animals were killed. An aliquot of blood was centrifuged at 4°C for 15 min at 3,000 rpm. The serum and plasma were frozen at −80°C until assays were conducted. IgM, IgG, IgA, and complement C3 and C4 were determined in serum by nephelometry (Immage Beckman Coulter, Brea, CA). Plasma corticosterone (7.1%) concentrations were assayed by a double antibody radioimmunoassay method specific for rats and mice using a commercial kit (MP Biomedicals, Orangeburg, New York). The sensitivity of the assay was 0.25 ng/ml.

Spleen Weight and Lymphocyte Counts

Spleen and axillary lymph nodes were removed after the death, and the spleen weight was immediately measured. For the examination of cell numbers in spleen and axillary lymph nodes, single cell suspensions were prepared by pressing them through 400-μm sterile nylon mesh. Cells were counted in a Neubauer chamber with the aid of a microscope by the same previous experimenter.

Statistical Analysis

Values are expressed as means ± SE. One-way ANOVA was performed followed by Duncan's multiple range test. The level of statistical significance was set at $P \leq 0.05$.

RESULTS

Circulating White Blood Cell Count (Leukocyte Cell Differentials)

Total blood leukocytes. ANOVA test revealed significant differences among groups [F (6,61) = 4,65; $P < 0.001$]. Post hoc testing indicated that SR for 21 days reduced total leukocytes compared with the control group ($P < 0.01$). PSD for 96 h increased leukocyte population in relation to the PSD 24h ($P < 0.03$) and SR 21d ($P < 0.001$) groups. Rats subjected to recovery from PSD 96h had significantly higher concentrations than those of the PSD24R ($P < 0.02$) and SR21R ($P < 0.02$) groups (Fig. 1*A*).

Monocytes. At SR 21d, these cells were significantly decreased when compared with those of the PSD 96h group ($P < 0.01$) (Fig. 1*B*). Monocyte number significantly increased after recovery from PSD 96h compared with CTRL ($P < 0.004$), PSD24R ($P < 0.001$), and SR21R ($P < 0.001$), as revealed by ANOVA [F (6,61) = 4.79; $P < 0.001$] followed by Duncan's multiple range test.

Neutrophils. Neutrophil values are depicted in Fig. 2*A*. An ANOVA test indicated significant differences among groups [F (6,61) = 8.61; $P < 0.001$]. Duncan's multiple range test revealed that rats subjected to PSD or SR did not exhibit significant alterations compared with control rats. However, recovery from PSD 96h had significantly higher values than those observed in all other groups: CTRL ($P < 0.001$), PSD 24h ($P < 0.001$), PSD 24R ($P < 0.001$), PSD 96h ($P < 0.01$), SR 21d ($P < 0.001$), and SR 21R ($P < 0.001$). Neutrophils were decreased in the SR 21d group compared with the PSD 96h group ($P < 0.007$).

Lymphocytes. Sleep deprivation for 24 h ($P < 0.001$) and SR for 21 days ($P < 0.001$) significantly reduced the number of lymphocytes compared with the control group [F (6,61) = 4.00; $P < 0.001$]. The PSD 96h group yielded significantly higher values than those observed in the PSD 24h ($P < 0.007$) and SR 21d ($P < 0.008$) groups. Upon recovery, lymphocytes

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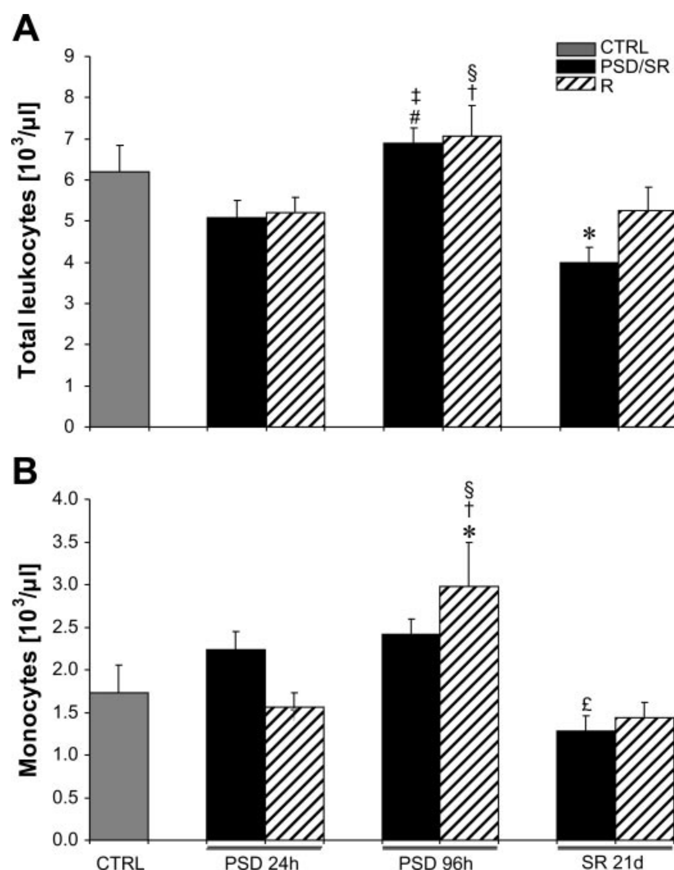

Fig. 1. Mean values ± SE of total leukocytes (*A*) and monocytes (*B*) in home-cage control (CTRL) group and paradoxical sleep-deprived (PSD) groups for 24 and 96 h and sleep-restricted (SR) groups for 21 days, and their recovery (R) periods. *Different from the control group; #different from the PSD 24h group; †different from the PSD24R group; £different from the PSD 96h group; ‡different from the SR 21 day (SR 21d) group; §different from the SR21R group.

were significantly higher compared with respective SR 21d ($P < 0.02$) as depicted in Fig. 2*B*.

IgM, IgG, IgA, and Complement C3 and C4

SR for 21 days ($P < 0.002$), as did their recovery period ($P < 0.001$), significantly increased the IgM concentration compared with control group [F (6,64) = 6.9375; $P < 0.00001$], as indicated in Fig. 3*A*. The SR 21d group also presented increased concentrations of IgM compared with the PSD 24h ($P < 0.001$) and PSD 96h ($P < 0.001$) groups. Concentrations of IgM in the SR21R group differed from those of the PSD24R ($P < 0.003$) and PSD96R ($P < 0.002$) groups.

Complement C3 was higher after PSD24R ($P < 0.002$), PSD 96h ($P < 0.01$), PSD96R ($P < 0.001$) compared with the control group. PSD 96h yielded significantly higher values than collected from the PSD 24h ($P < 0.01$) and SR 21d ($P < 0.006$) groups. PSD24R also increased compared with the PSD 24h ($P < 0.002$) group [F (6,62) = 9.1761; $P < 0.00001$] (Fig. 3*B*). C3 were significantly lower on SR21R group compared with PSD24R ($P < 0.001$) and PSD96R ($P < 0.001$).

No significant differences among groups were found in the serum concentrations of IgG, IgA, and Complement C4 (data not shown), as indicated by ANOVA test.

Spleen Weight

ANOVA test revealed significant differences among groups [F (6,64) = 4.68; $P < 0.001$]. Duncan's multiple range test indicated that SR for 21 days significantly reduced spleen weight compared with all other groups: CTRL ($P < 0.001$), PSD 24h ($P < 0.02$), PSD 24R ($P < 0.001$), PSD 96h ($P < 0.02$), PSD96R ($P < 0.001$), and SR21R ($P < 0.02$) (Fig. 4).

Number of Lymphocytes in Spleen and Lymph Nodes

As indicated by the ANOVA test [F (6,63) = 6.49; $P = 0.0001$], the number of lymphocytes in the axillary lymph nodes significantly increased after recovery from PSD for 24 h compared with all groups: CTRL ($P < 0.001$), PSD 24h ($P < 0.001$), PSD 96h ($P < 0.01$), PSD96R ($P < 0.001$), SR 21d ($P < 0.001$), and SR21R ($P < 0.001$) (Fig. 5). ANOVA test revealed no significant differences among groups in the number of cells in the spleen ($P > 0.05$).

Corticosterone

Corticosterone concentrations increased significantly after PSD for 96 h compared with all other groups: CTRL ($P < 0.01$), PSD 24h ($P < 0.01$), PSD24R ($P < 0.001$), PSD96R

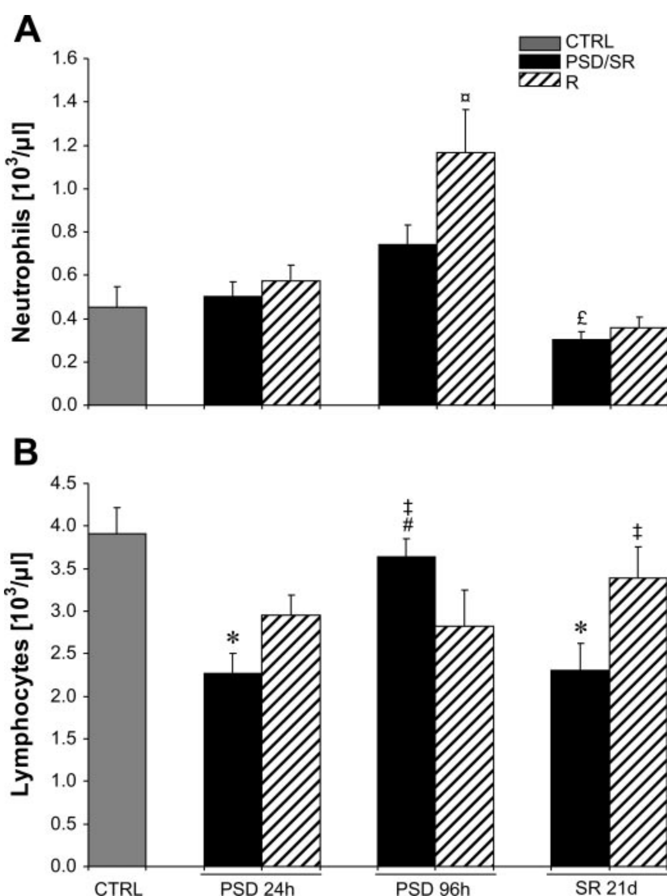

Fig. 2. Mean values ± SE of neutrophils (*A*) and lymphocytes (*B*) in CTRL group and PSD groups for 24 and 96 h and SR groups for 21 days, and their R periods. *Different from the control group; #different from the PSD 24h group; £different from the PSD 96h group; ‡different from the SR 21d group; ¤different from all groups.

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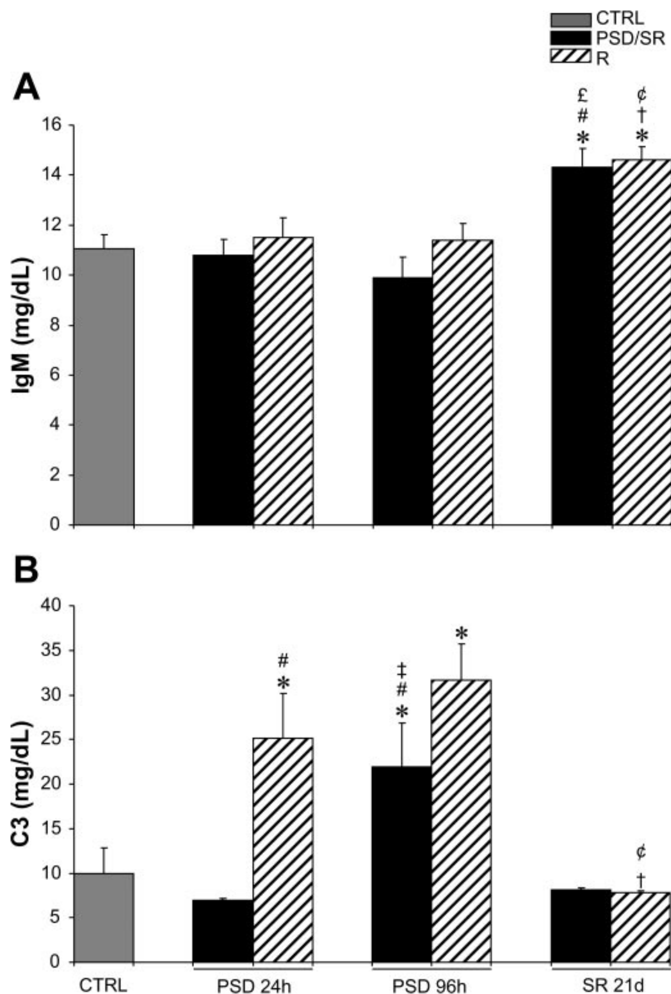

Fig. 3. Mean values ± SE of IgM (*A*) and C3 (*B*) in the CTRL group and PSD group for 24 and 96 h and SR group for 21 days, and their R periods. *Different from the control group; #different from the PSD 24h group; †different from the PSD 24R group; £different from the PSD 96h group; ¢different from the PSD 96R group; ‡different from the SR 21d group.

($P < 0.001$), SR 21d ($P < 0.01$), and SR21R ($P < 0.001$), as indicated by ANOVA [F (6,46) = 5.57; P = 0.0002] (Fig. 6).

DISCUSSION

The SD procedures as used in the present study were sufficient to produce marked alterations in almost all of the immune parameters that were examined as indicated in Table 1. Lymphocyte count decreased after PSD for 24 h, whereas after 96 h of PSD, increases in C3 and corticosterone concentrations were observed. The major alterations occurred in the SR paradigm: spleen weight, total leukocytes, and lymphocytes decreased in relation to the control group. Moreover, an increase in IgM was also observed in the SR group. When recovery sleep was evaluated, C3 concentrations increased and lymphocyte count in axillary lymph nodes grew when rats had rebound sleep after PSD for 24 h. Moreover, the recovery group of 96 h of sleep loss had increased number of neutrophils, while presenting lower corticosterone concentrations. Higher spleen weight and number of blood lymphocytes were observed in the SR21R group. Collectively, the alterations that occurred during SD suggest only minor changes of nonspecific immune parameters on the short-term periods and an impairment of cellular response during long-term SR.

The number and proportions of leukocytes in the blood and immune organs provide an important representation of leukocyte distribution in the body and of the state of activation of the immune system. A previous study showed that alterations in immune circulating cells occurred when rats were subjected to chronic SD by the disk-over-water method (14). However, the methodology that was adopted in that study had the animal move about to maintain wakefulness, and physical exercise was added as a variable of that investigation.

Leukocytosis induced by SD has been a consistent finding in human sleep loss studies since at least 1925, sometimes composing the only physiological finding (20). In contrast, other reports with humans corroborate our findings with animal experimentation and did not show significant differences in circulating monocytes and neutrophils after SD. In a human study using SD for 48 h, these leukocytes did not show any significant difference during the SD procedure (28).

Consistent with our findings, previous studies have shown decreased circulating lymphocytes counts after SD (5, 31). The spleen is a critical immune organ, which has been shown to increase in size after immune activation due to B and/or T cell differentiation and proliferation. Thus, post mortem spleen weights provide a rough estimate of the degree of immune activation (13). To our knowledge, few studies have focused on spleen weight and number of lymphocytes in spleen and axillary lymph nodes related to sleep and/or SD. In this sense, our study demonstrated that only chronic SR resulted in reduction in spleen weight, which was reverted after recovery. In common, these studies raise the hypothesis that the degree, the methodology, and the duration of SD are important in determining its effects on immune function.

Ig and complement levels in serum are part of the data used to assess the integrity of immune system functioning and are essential in the understanding of the impairment and restorative processes occurring during wakefulness and sleep. In this context, the current data are in agreement with previous findings demonstrating an increase in C3 concentration in acute SD

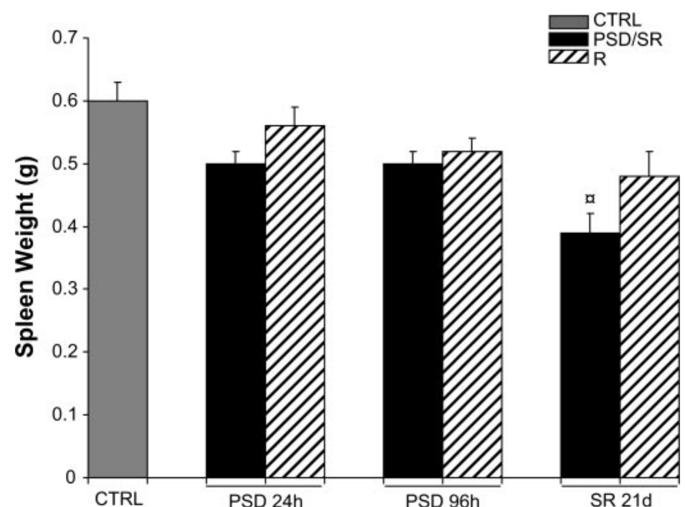

Fig. 4. Mean values ± SE of spleen weight in CTRL groups and PSD groups for 24 and 96 h and SR groups for 21 days, and their R periods. ¤Different from all groups.

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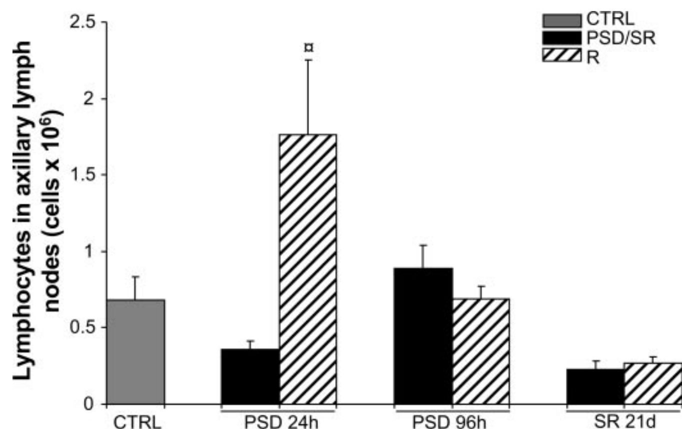

Fig. 5. Mean values ± SE of the number of lymphocytes in the axillary lymph nodes in CTRL groups and paradoxical PSD groups for 24 and 96 h and SR groups for 21 days, and their R periods. ¤Different from all groups.

humans (17) and that IgM was the first Ig to increase in chronic SD rats (14). No differences between groups were found in the serum concentrations of IgG, IgA, and complement C4.

Studies have shown that alteration in leukocyte distribution is mediated by hormones released by the adrenal gland, like corticosterone. It may be worth noting that most stress conditions, which are found to be immunoenhancing, involve acute stress, and those that are found to be immunosuppressive involve chronic stress (with the effects of stress on leukocyte distribution being an important factor to be taken into account) (9, 12, 32, 35). SD is an inherently stressful procedure, and it may not be possible to completely extricate the effects of SD from general nonspecific stress effects (1, 3). There is some evidence that there are reciprocal relationships between immune function and increased hypothalamic-pituitary-adrenocortical axis activity in depression (26) and in stress response (10, 11, 12).

A question remained as to whether the observed alteration in immune parameters was caused by sleep loss or by stress inherent to the present methodology. Chronic SR for 21 days by the modified multiple platform method did not alter the concentration of corticosterone but did cause the most significant alterations in immune cells, demonstrating that methodology stress did not lead to those alterations, which were attributed to sleep loss per se. Of note is that although some immune parameters recovered their normal values rather rapidly, others remained altered, indicating that sleep normalization (i.e., recovery) is not the sole factor that leads to the recovery of homeostasis.

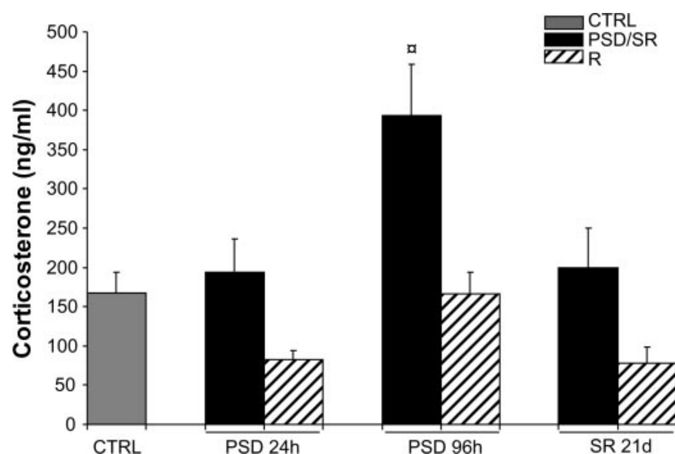

Fig. 6. Mean values ± SE of corticosterone concentrations in CTRL and PSD groups for 24 and 96 h and SR groups for 21 days, and their R periods. ¤Different from all groups.

Table 1. *Summary of obtained results of PSD groups for 24 and 96 h and SR groups for 21 days compared with control and between R and respective PSD/SR groups*

	PSD 24 h	PSD 96 h	SR 21 Days
Control			
Total blood leukocytes			↓
Monocytes			
Neutrophils			
Lymphocytes	↓		↓
IgM			↑
C3		↑	
Spleen weight			↓
Number of lymphocytes in lymph nodes			
Corticosterone		↑	
	PSD24R	PSD96R	SR21R
R and PSD/SR Groups			
Total blood leukocytes			
Monocytes			
Neutrophils		↑	
Lymphocytes			↑
IgM			
C3	↑		
Spleen weight			↑
Number of lymphocytes in lymph nodes	↑		
Corticosterone		↓	

PSD, paradoxical sleep deprived; SR, sleep-restricted; R, recovery groups.

Although SD is considered a risk factor for human disease, and some specific health impairments have been linked to SD, it is a condition that increases progressively in current society, in which the population tends to sleep less due to social pressure. Thus, sleep fragmentation associated to the SD method is a condition that mimics the human lifestyle of restricted sleep time and prolonged wakefulness, making that animal model ideal for studies of sleep and sleep loss.

In the present study, however, the two experimental methods used showed distinct effects in immune system: PSD for 24 h caused only a decrease of blood lymphocytes and PSD for 96 h resulted in an increase of C3 concentration. In contrast, SR for 21 days decreased total leukocytes, lymphocytes, and spleen weight and increased unspecific IgM production. In conclusion, the present findings indicate that sleep and sleep loss play an important role exerting differing physiological influence in the modulation of immunity. Our data suggest that although acute sleep loss as that described in our PSD protocol causes minor alterations in some immune parameters, prolonged SR like that observed in a modern population on a daily basis, causes impairment of systemic circulation of leukocytes and quite possibly increases the susceptibility to infectious diseases.

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GRANTS

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