

Effects of sleep deprivation on wound healing

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SUMMARY Sleep deprivation is widely regarded as a stressor and has been shown to have significant effects on host defences. Severely sleep-deprived rats develop lesions on their paws and tails, suggesting possible deficits in the healing process. The purpose of this study was to assess the impact of rapid eye-movement (REM) sleep deprivation (RSD) on wound healing in a rat model. Male dark-hooded Long-Evans rats, 2–4 months old, were subjected to dorsal application of two sterile punch biopsies, each 3.5 mm in size. Biopsies were performed either immediately before or immediately after 5 days of sleep deprivation. Wound healing in REM sleep-deprived animals was compared with home cage control and yoked control animals. RSD did not produce differences in the rate of healing, regardless of the timing of the biopsy punch. RSD does not appear to have significant effects on wound healing and thus appears to act differently from other types of stressors on wound healing.

KEYWORDS rapid eye-movement sleep, skin, sleep deprivation, stress, wound healing

INTRODUCTION

Sleep is generally assumed to be conducive to healing. Indirect evidence that suggests sleep may be important for healing includes the observations that protein synthesis, cell division and growth hormone release are increased during sleep (Adam and Oswald, 1983; Sassin *et al.*, 1969). Sleep deprivation, on the other hand, has significant effects on a number of parameters that could negatively impact the healing process. Systemic effects of total sleep deprivation include reductions in growth hormone secretion (Van Cauter *et al.*, 1992), increased activation of the sympathetic nervous system (Irwin *et al.*, 1999), activation of the hypothalamic–pituitary–adrenal (HPA) axis (Leproult *et al.*, 1997; Spiegel *et al.*, 1999) and alteration of host defence responses (Benca and Quintans, 1997; Marshall and Born, 2002; Opp and Toth, 2003).

Several of the long-term effects of chronic sleep deprivation in rats suggest specific breakdown in skin and mucosal barrier functions. Rats subjected to prolonged total sleep deprivation, rapid eye-movement (REM) sleep deprivation (RSD), or slow-wave sleep deprivation develop ulcerative and hyperkeratotic

lesions on their paws and tails (Gilliland *et al.*, 1989; Kushida *et al.*, 1989); the etiology of these lesions remains unknown, although they do not appear to be caused by an infectious process. Furthermore, rats at the near-terminal stage of total sleep deprivation have increased rates of bacteremia in comparison with controls (Everson, 1993; Everson and Toth, 2000). It has been suggested that systemic infection in sleep-deprived rats may be because of dysfunction of gut mucosa, resulting in increased translocation of bacteria from the gastrointestinal tract to the blood (Bergmann *et al.*, 1996a; Everson and Toth, 2000). A study in humans has suggested that short-term sleep deprivation may affect skin barrier function (Altemus *et al.*, 2001). Forty-two hours of wakefulness caused an increase in plasma interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), and natural killer (NK) cell activity as well as a decrease in skin barrier function recovery, suggesting that sleep deprivation-induced changes in proinflammatory cytokines may be responsible for changes in host defenses. Sleep deprivation has been found to affect host defense functions in some but not all paradigms, and the effects—when present—appear to be specific to the type of defense reaction (Benca and Quintans, 1997). For example, some types of immune responses may also be enhanced by sleep deprivation, as in the case of tumor regression (Bergmann *et al.*, 1996b).

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Establishing models to demonstrate the link between sleep deprivation and important clinical outcomes is necessary to better understand the effects of sleep deprivation on physiological systems. This is particularly necessary as effects of sleep deprivation on host defenses are not uniform, but likely vary in relation not only to the specific immune challenges (e.g. bacteria, viruses, tumors) but also to the nature of possible insults to host defenses (e.g. wounds, burns, stress). The purpose of the present study was to determine if RSD might have an impact on skin healing, as spontaneous, non-healing skin lesions are a hallmark of severe sleep deprivation in rats and are produced by both total sleep deprivation as well as RSD (Rechtschaffen *et al.*, 1989).

From a clinical perspective, sleep disruption frequently occurs postoperatively or following an acute injury and could thus affect wound healing. A previous study failed to document significant effects of 72 h of total sleep deprivation on cellular and biochemical markers of wound healing in rats (Landis and Whitney, 1997). In that study, however, sleep deprivation began 7 days after the wounds were produced. As healing is usually well established by 1 week, it is possible that the failure to see sleep deprivation-induced changes could have been related to the timing of the deprivation. Furthermore, sleep disruption in clinical situations is most likely to occur immediately following surgery or an injury. In this study, we sought to determine whether RSD in the days immediately before or after wound production would alter the rate of healing.

METHODS

Male dark-hooded Long-Evans rats, approximately 300–400 g and 2–4 months of age, were obtained from Charles River (Portage, Michigan). Rats were maintained on a 12 h : 12 h light : dark schedule at a temperature of $23 \pm 1^\circ\text{C}$. The University of Wisconsin Research Animal Resources Center approved the protocol.

Sleep deprivation

For each experiment, animals were divided into three groups: home cage controls (H), apparatus controls (C), and RSD animals (D). H animals remained in individual housing in standard cages throughout each experiment.

C and D rats were housed in a large tank (173 × 83 cm) partitioned with clear acrylic into six sections (54 × 37 × 44 cm), such that the rats were in visual contact with one another. C and D rats were alternated in the compartments so that C rats were next to D rats. They were housed on platforms, the tops of which protruded approximately 3 cm above a water bath. The multiple platform method was used to achieve selective RSD (Coenen and van Luijtelaar, 1985). The platforms for the D animals were 6 cm in diameter, which is sufficient to allow for the occurrence of most non-REM sleep (Landis, 1996; Vogel, 1975) the muscle atonia, which accompanies REM sleep, makes REM bouts

impossible on small platforms, whereas H rats were housed on platforms 11 cm in diameter. Multiple platforms (three for D rats, two for C rats) were provided to each animal to minimize confinement effects. Animals stayed in the experimental apparatus for 5 days. At the end of the 5-day REM sleep deprivation period, C and D animals were returned to standard cages and housed individually.

All rats received biopsies according to the following procedure: rats were anesthetized with isoflurane. Skin of the back was shaved and disinfected with Betadine and alcohol 70%. Rats were given two full-thickness punch biopsies of 3.5 mm each with Sklar sterile punches. Biopsies were placed on either side of the spine at approximately the level of the front limbs. The biopsies were performed on one of two schedules, either immediately before 5 days of RSD ($n = 8$ per group), or after 5 days of RSD ($n = 12$ per group). H and C animals received biopsies at the same time as D animals.

Several animals were excluded from analysis because of clinically obvious complications. Ulcerative dermatitis associated with staphylococcal infection (Table 1) and accompanied by pruritus was the major complication and occurred in several animals (4 H, 5 C and 3 D). The infection was self-limiting in the majority of the cases. Cultures of the infections were all positive for *Staphylococcus*; most animals grew coagulase positive *Staphylococcus*, but coagulase negative and Beta hemolytic *Staphylococcus* growth occurred in a few animals as well. An additional six animals (3 H, 1 C, and 2 D) were removed from consideration before analysis to avoid confounding the results: four scratched enough to enlarge the initial wounds or to create new wounds; one persistently got bedding caught in the wound; and one injured its tail, the tip of which was then amputated. The decision to eliminate these subjects from the experiment was made by an individual blind to their identities. This left a total of 7 D, 8 C, and 7 H animals in the experiment where biopsies were performed prior to RSD and 8 D, 5 C and 7 H animals with biopsies performed after RSD.

Table 1 Length of time from biopsy punch until infection was suspected. Rats are listed by condition and timing of biopsy punch relative to RSD

Treatment	Schedule	Rat ID	Days
H	Biopsy first	12Z4	15
H	RSD first	13A8	10
H	RSD first	13J1	8
H	RSD first	13R8	15
C	RSD first	13A2	10
C	RSD first	13A7	5
C	RSD first	13D3	8
C	RSD first	13D7	8
C	RSD first	13J8	15
D	RSD first	13A4	5
D	RSD first	13R2	9
D	RSD first	13R7	9

Measurement of healing

Photographs (resolution = 1600 by 1200 pixels) were taken daily at approximately the same time in the room in which animals were housed to minimize handling. A bracket affixed to the camera with an opening to frame the wound was used to ensure that the distance from the lens to the wound was the same from day to day and from site to site. An outline was drawn (graphics tablet; WACOM, Inc., Vancouver, WA) around the photo of the wound; the area of the wound was calculated using image analysis software (Image/J, <http://rsb.info.nih.gov/ij/>). The size was then transformed from area to radius (R) before further calculation was done. The initial size of the wound was determined from a photograph taken approximately 5 min after the second punch. Degree of healing is expressed as percentage healed [$1 - (R/R_{\text{initial}})$] in order to minimize the effect of slight differences in the initial size of the wounds. Healing was considered complete when the surface of the wound was covered by intact epidermis (Fig. 1). The

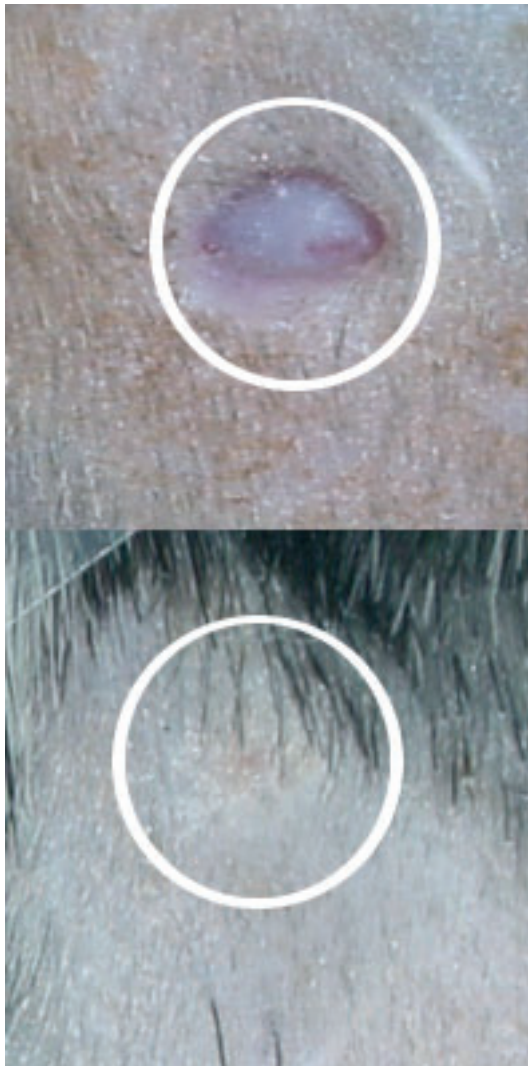


Figure 1. Example of the area containing a wound immediately after biopsy punch and the same area when healing was deemed complete.

drawing of the wound outline, the recording of the size of the wound and the determination of whether healing was complete were performed by an individual unaware of the identities of the animals or the groups to which they belonged. The length of time was counted from the date and time of the biopsy punch until date and time of the first observation that a wound was healed. The 'time to healing' was estimated for each animal as the average of the times for the two wounds.

Statistical tests

The changes in wound size were compared using a linear mixed-effects model, testing for the effects of group (H, C, or D) and schedule (biopsy before REM deprivation versus biopsy after REM deprivation) on the slope using R (version 1.9.1, R Foundation for Statistical Computing, Vienna, Austria). These comparisons were based on the daily average of the two wounds per animal. ANOVAS were also performed on overall time to heal of the individual wounds. The comparisons were repeated using the nonparametric Kruskal–Wallis test. These tests were performed using SPSS (ver. 10.0.7, SPSS Inc., Chicago, IL, USA).

RESULTS

In the first experiment, rats received 5 days of RSD immediately following a full-thickness skin biopsy. Time to healing (i.e. the mean length of time for complete healing of the two wounds on each rat) ranged from 9 to 15 days and did not differ ($F_{2,20} < 1$) among D, C, and H rats (Fig. 2); power calculations indicated an 80% chance of detecting a difference as small as 2 days. The tests were repeated using the minimum time for either of the two wounds to heal, and again with the time taken for both wounds to heal completely; both analyses provided similar results. Power was somewhat less for these tests (a 3-day rather than a 2-day difference could be detected with $\beta = 0.8$), as would be expected from the higher variability inherent in measuring single wounds.

In the second experiment, D rats were first sleep deprived for 5 days and received the biopsies at the end of the deprivation period. Once again, no significant differences ($F_{2,22}$, $P = 0.356$) in time to healing were observed across D, C and H groups of animals (Fig. 2). As in the first experiment, a 2-day difference could have been detected with a $\beta = 0.8$. Healing times were similar to the first experiment and ranged from 10 to 15 days. Once again, we repeated the analysis on the earlier and later healing of the wounds and found similar results ($P > 0.25$) across conditions.

To ensure that the parametric results represented the typical outcome for the group, we repeated all of the above comparisons using ranks of time to healing. The results were similar, indicating no significant difference among groups (Kruskal–Wallis, $P > 0.25$ in all comparisons).

The animals healed at a similar rate (i.e. the slope of percentage healed by time) across groups with no significant

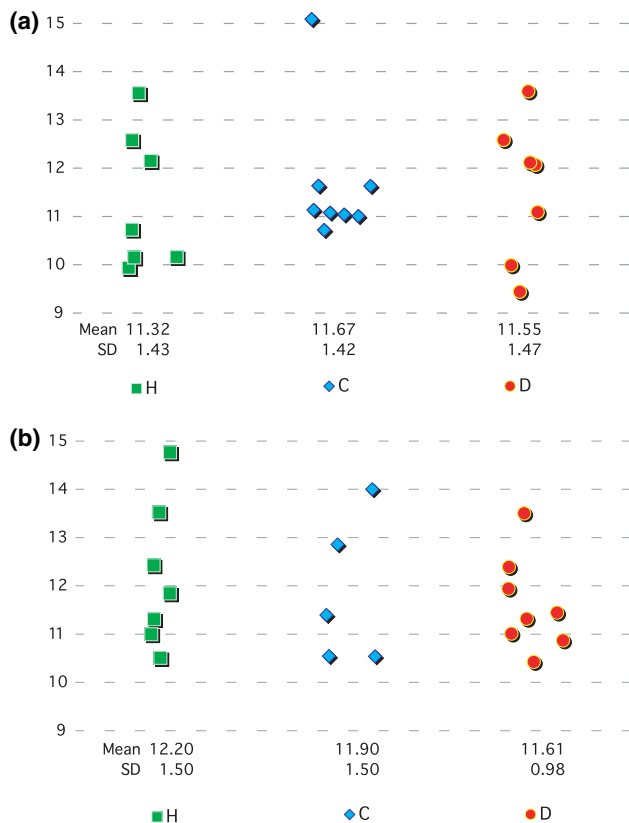


Figure 2. Number of days from biopsy punch to healed wound. Each point shows the length of time needed for an animal to heal (the average of two biopsy punch wounds for each animal.) The animals in which the biopsy punch preceded REM sleep deprivation are graphed in (a) and those in which the REM sleep deprivation preceded the wound are shown in (b).

differences by group, or any interaction between group and schedule ($F < 1$). The difference in the rate of healing between the two biopsy schedules reached significance ($P < 0.01$) without respect to group; overall, animals receiving the biopsy after RSD healed at a slower rate than animals that began their healing during the period of RSD. The same analysis of the raw radius of the wounds (without respect to initial size) by time indicated a significant difference in slope by group, but no difference by schedule. Inspection suggested that the shallower slope among the H controls was due to a single animal (see Fig. 2). Without this animal there were no significant differences in slopes of radius across time by group or by group and schedule. With the removal of this H control outlier that took an unusually long time to heal, the difference in the rate of healing (scaled to initial wound size) by schedule (biopsy before or after RSD) disappeared.

Although 5 days of RSD did not seem to influence the time required for overall healing, we sought to determine whether there might be effects of sleep deprivation at the beginning of the healing process. In normal healing, a wound generally becomes slightly larger during the first few days before it starts to contract. D, C and H groups did not differ in wound size

closure during the first 3 days or proinflammatory phase of healing ($P > 0.5$).

Several animals were removed from the analyses, primarily because of superimposed infections that were clinically evident. There was no indication from these data for a causal association between sleep and the occurrence of infection. In no case was the rate of infection for D rats greater than that for C or even H rats (see Table 1). There was a higher rate of infection among rats in the experiment that had the biopsy after RSD rather than before RSD; this was as true for the H rats whose treatment was identical between experiments as for the C and D rats. Infected animals showed delayed healing in comparison with those without obvious infection, but did not show a relationship between this delay and RSD.

DISCUSSION

Results of this study suggest that 5 days of RSD either immediately preceding or following wound formation had no significant effect on the initial phases of healing in rats. Specifically, there was no evidence of any change in the rate of wound closure at any time from the initiation of the wound to epithelialization.

We sought to investigate the effects of short-term RSD on wound healing because rats deprived of total sleep or REM sleep develop non-healing lesions on their paws and tails (Gilliland *et al.*, 1989; Kushida *et al.*, 1989), suggesting a possible breakdown in skin barrier function. We postulated, however, that RSD might either delay or even enhance healing time as sleep loss can either impair or enhance host defenses, depending on the nature of the response measured (Benca and Quintans, 1997; Opp and Toth, 2003).

Wound healing is a complex process that is comprised of several overlapping phases (Bello and Phillips, 2000; Singer and Clark, 1999), and sleep loss might be expected to have an impact on various aspects of this process. Immediately after wounding, coagulation occurs to produce hemostasis. Within minutes, inflammation begins as a variety of cell types migrate into the wound. Initially neutrophils and monocytes phagocytize bacteria and cell debris. Later, fibroblasts migrate to the site to deposit collagen and form new tissue. The processes of inflammation and cell proliferation are regulated by polypeptide growth factors and cytokines, many of which can be affected by sleep deprivation. For example, sleep loss leads to decreased secretion of GH (Van Cauter *et al.*, 1992), and increased release of proinflammatory cytokines such as IL-1 and TNF (Krueger *et al.*, 1998). Sleep deprivation may also influence healing through effects on tissue perfusion. Total sleep deprivation may cause a slight vasodilation (Zenko *et al.*, 2000), which could theoretically cause increased tissue perfusion. Thus sleep deprivation could influence wound healing through a number of mechanisms that could have both enhancing and/or impairing effects on the overall process.

Two previous studies have looked at the effects of sleep deprivation on healing or skin barrier function. Landis and Whitney assessed wound healing at a cellular level by inserting

expanded polytetrafluoroethylene tubing into the subcutaneous tissue of rats (Landis and Whitney, 1997). They used a similar sleep deprivation technique (single flowerpot inverted in water rather than the multiple flowerpot method used in this study), but focused on later stages of wound healing; rats were sleep deprived for 72 h (rather than 120 h as in the present study), beginning 7 days after wound formation. No differences were reported between RSD or control rats in numbers of macrophages, granulocytes or fibroblasts or amounts of protein, DNA or hydroxyproline. Although these data demonstrate that later stages of healing were not affected by RSD, it is possible that sleep deprivation might have had greater effects on the early, inflammatory phase of healing.

A study by Altemus *et al.* (2001) in fact assessed the effects of 42 h of sleeplessness versus two other stressors on the immediate recovery of skin barrier function in healthy women following repeated tape stripping of the skin. Both sleep deprivation and an interview stress paradigm resulted in a significant delay in the recovery of skin barrier function as measured by transepidermal water loss; exercise stress appeared to have no effect. This study also demonstrated differing effects of the psychological (interview) stress versus sleep deprivation on neuroendocrine and immune parameters. Psychological stress led to increases in plasma cortisol, norepinephrine, IL-1 β , IL-10, TNF- α , circulating NK cells and NK activity; whereas sleep deprivation was associated with increased plasma IL-1 β , TNF- α and NK activity. The third stressor, exercise, led to increased NK activity. Based on these data, the authors postulated that deleterious effects on skin barrier function might have been due to elevations in the proinflammatory cytokines TNF- α and IL-1 β .

The present study focused on the early stages of healing, to the point of initial wound closure. Sleep deprivation was performed during the initial phase of healing, as well as immediately prior to the start of healing. Similar to the Landis and Whitney (1997) study, we found no differences in the rate of healing, suggesting that neither the early inflammatory nor later proliferative phases of healing appear to be significantly affected by REM sleep deprivation. It is not possible to say whether the initial hours of skin recovery were influenced by sleep deprivation, but even if they were, this did not appear to influence the overall course of healing in a significant manner.

Sleep deprivation effects on healing appear to differ from the effects of other stressors. Several studies in humans and rodent models have shown delayed healing in response to stress. Kiecolt-Glaser *et al.* (1995) demonstrated that the stress of caring for a relative with Alzheimer's disease significantly delayed healing of a skin biopsy wound by 24% (9.4 days) in comparison with age-matched controls. Mucosal wounds in healthy dental students healed more slowly (40% or 3 days longer) when they were produced during a period of examination stress than during summer vacation (Marucha *et al.*, 1998). Finally, healing of punch biopsy wounds in mice was delayed by 27% (3.1 days) with restraint stress (Padgett *et al.*, 1997). It has been postulated that stress-induced elevations of glucocorticoids mediate the delays in healing; (Padgett *et al.*

(1997) demonstrated that glucocorticoid receptor blockade reversed the healing delays in mice. Glaser *et al.* (1999) found that women with higher stress levels had elevations in salivary cortisol and reduced levels of proinflammatory cytokines (IL-1 α and IL-8) at the site of an induced skin blister. He has suggested that stress may thus delay wound healing through reduction of local proinflammatory cytokines at the wound site, at least in part related to elevations in glucocorticoid levels.

Based on the magnitude of the delays in biopsy healing in humans and mice subjected to various stressors, it is possible to conclude that RSD does not have a comparable effect on healing, as the present study had adequate power to detect differences at least as small as 2 days between groups ($\beta = 0.8$). In comparison with other types of stressors, sleep deprivation may have different effects on various neuroendocrine parameters, such as the HPA axis. For example, studies of chronic sleep deprivation in rats have shown that ACTH and cortisol levels do not increase during chronic sleep deprivation (Rechtschaffen and Bergmann, 1995; Rechtschaffen *et al.*, 1989). However, a recent study in humans by Leproult *et al.* (1997) suggested that one night of partial or total sleep deprivation led to an increase in serum cortisol levels during the next evening, but levels were not chronically elevated. The authors concluded, however, that sleep deprivation did not necessarily stimulate the HPA axis, like other stressors, but rather decreased the rate of recovery and/or the reduction of activity. There was, however, no change in the linear rate of healing among the present groups following RSD, and initial delay was most prominent in the control rather than the RSD group.

Although it has been suggested that the platform method of RSD, including variations using multiple platforms, may produce stress effects, including activation of the HPA axis (Suchecki *et al.*, 1998; Vogel, 1975), it is not clear whether these effects are due to the loss of REM sleep or stress related to the methods used to produce RSD, as both may increase HPA axis activity. In the present study, the potential contributions from other sources of stress were probably reduced by the use of multiple flowerpots for each animal that minimized confinement stress, and the partitioned tank that minimized isolation stress. Furthermore, both C and H rats were used as comparator groups for the RSD animals, to determine the effects of RSD above and beyond the stressful effects of the apparatus. In this study, RSD clearly did not act like other types of stressors in terms of effects on wound healing and, for that matter, there was no discernable effect of the apparatus on wound healing, as evidenced by the similar rates of healing in C and H rats.

One caveat of this study is that we deprived rats of REM sleep rather than total sleep. As total sleep deprivation may have somewhat different effects and/or produce disturbances in physiological parameters more quickly (Rechtschaffen *et al.*, 1989), it is possible that longer-term or total sleep deprivation could have a more significant impact on healing processes.

A number of animals were prospectively excluded from analyses during the study because of clinically apparent infections. Interestingly, all but one were from the study in which RSD was performed prior to wounding, but there was no increased risk of infection in D or C rats in comparison with H controls. This suggests that the group of animals used for this experiment, which was obtained from the supplier at a different time from the animals used in the experiment in which the biopsy was performed prior to RSD, might have had an increased susceptibility to infection that was exacerbated by the biopsy. There is no suggestion that RSD had a specific effect in promoting infection, however.

The results of this study, which suggest that even 5 days of RSD do not significantly influence the rate of wound healing, are relevant to clinical situations such as postoperative and post-traumatic recovery and suggest that sleep disturbance is unlikely to delay wound healing. Several studies have documented significant sleep disruption in hospitalized patients in burn units, intensive care units, and following surgery (Cooper *et al.*, 2000; Freedman *et al.*, 1999; Rose *et al.*, 2001). There have been no studies assessing the effects of sleep disturbance on healing in post-surgical or burn patients, however, although it might be expected that sleep should be beneficial in these groups of patients. For example, burn patients often show symptomatology similar to that reported in sleep-deprived rats, including a catabolic state, thermoregulatory abnormalities and presence of opportunistic infections (Rose *et al.*, 2001). Furthermore, sleep deprivation leads to decreased levels of growth hormone (Everson and Crowley, 2004), and administration of exogenous growth hormone may be beneficial in promoting healing in burn patients; these findings, along with the growing literature showing important relationships between sleep and response to infection (reviewed in (Opp and Toth, 2003), suggest possible mechanisms for beneficial effects of sleep on the healing process. It is important to note that the failure to detect significant effects of RSD on wound healing in the present study could be related to species-specific differences; rodents may be less sensitive to the effects of sleep loss on skin healing. Alternatively, mild-to-moderate sleep loss may not be sufficient to impair wound healing. Further studies in clinical settings as well as in animal models are needed to understand the role of sleep in modulating clinically relevant aspects of host defense functions.

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