Dose-dependent effects of endotoxin on human sleep

JANET MULLINGTON, CARSTEN KORTH, DIRK M. HERMANN, ARMIN ORTH, CHRIS GALANOS, FLORIAN HOLSBEO, AND THOMAS POLLMÄCHER
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Mullington, Janet, Carsten Korth, Dirk M. Hermann, Armin Orth, Chris Galanos, Florian Holsboer, and Thomas Pollmächer. Dose-dependent effects of endotoxin on human sleep. Am J Physiol Regulatory Integrative Comp Physiol 278: R947–R955, 2000.—The role of the central nervous system in the host response to infection and inflammation and modulation of these responses by the hypothalamic-pituitary-adrenal system are well established. In animals, activation of host defense mechanisms increases non-rapid eye movement (NREM) sleep amount and intensity, which, in turn, are thought to support host defense, or the body’s ability to defend itself against challenges to its immune system. In humans, the evidence is conflicting. Therefore, we investigated the effects of three placebo-controlled doses of endotoxin on host response, including nocturnal sleep in healthy volunteers. Administered before nocturnal sleep onset, endotoxin dose dependently increased rectal temperature, heart rate, and the plasma levels of tumor necrosis factor (TNF-α), soluble TNF receptors, interleukin (IL)-1 receptor antagonist, IL-6, and cortisol. The lowest dose reliably increased circulating levels of cytokines and soluble cytokine receptors, but it did not affect rectal temperature, heart rate, or cortisol. This subtle host defense activation increased deep NREM sleep amount, often referred to as slow-wave sleep (stages 3 and 4), and intensity (delta power). Conversely, the highest dose of endotoxin disrupted sleep. Whereas it is well established that the endocrine and thermoregulatory systems are very sensitive to endotoxin, this study shows that human sleep-wake behavior is even more sensitive to activation of host defense mechanisms.

In recent years, empirical evidence has accumulated suggesting that the central nervous system (CNS) and periphery are engaged in a reciprocal dialogue supporting host defense. Peripherally released cytokines relay messages to the CNS via active transport mechanisms, vagal afferents, and/or circumventricular organs, leading to neuroendocrine activation and fever (12, 36, 37). In humans, administration of purified endotoxin leads to activation of the hypothalamic-pituitary-adrenal (HPA) system (32, 35, 42). The HPA system and its sleep-related hormones play an important role in sleep-wake regulation (for reviews, see Refs. 8, 41), and one of the most complex responses of the CNS to infection is altered sleep-wake behavior. We conducted the present study to systematically characterize the dose-dependent effects of endotoxin on traditional host response parameters (temperature, heart rate, leukocyte counts, cytokine, and neuroendocrine parameters) and on sleep.

In rodents and rabbits, bacteria, fungi, viruses, and their pathogenic components activate the primary host response and increase non-rapid eye movement (NREM) sleep amount and electroencephalogram (EEG) delta power, suggesting an enhanced sleep intensity (for reviews, see Refs. 20, 27, 44). Rapid eye movement (REM) sleep is found to be slightly suppressed or not affected. These changes in sleep parameters occur rapidly and may be mediated by inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β (reviewed in Ref. 21). It is suggested that deep NREM sleep is linked to body restitution (1), and its increase during infection is suggested to support host defense (44).

In humans, the data on the influence of naturally occurring infections on sleep are scarce and conflicting (reviewed in Ref. 31). The present knowledge about the effects of host defense activation on human sleep-wake behavior relies mainly on studies using experimental endotoxemia. This model is well established for the investigation of early events during bacterial infection and sepsis (reviewed in Refs. 5, 25). Whereas studies performed with healthy young volunteers consistently report various degrees of REM sleep suppression after the administration of endotoxin (2, 18, 19, 33), the effects on NREM sleep reported so far have been less consistent. A suppression of NREM sleep was found when relatively high doses of endotoxin that consistently induced flulike symptoms and prominent increases in rectal temperature were administered (18). After an evening administration of a mildly pyrogenic dose of endotoxin, the duration of light NREM sleep (stage 2) was increased (33), but not affected when the same dose was applied in the morning (19). So far, no consistent endotoxin-induced changes in human EEG delta power, an indicator of sleep intensity, have been found (33, 46). However, studies published to date have been performed with different endotoxin preparations, doses, and delays between administration and sleep onset, factors that may influence the response of sleep to an early immune challenge. This study clarifies discrepancies in the existing human literature by investigating dose-dependent effects of host challenge on sleep.
In the present study, we show that in healthy humans intravenous injection of endotoxin immediately before sleep onset in the evening may promote deep NREM sleep amount and intensity, or considerably disrupt sleep, depending on the dose applied. Furthermore, the sleep-wake system is more sensitive to the stimulation of cytokines involved in early host defense than is the HPA (as manifest by peripherally measured cortisol) or the thermoregulatory systems (rectal temperature).

METHODS

Subjects. Nineteen healthy males between the ages of 21 and 34 (mean 27.6 yr) participated in the study. All the volunteers provided an informed written consent. An independent ethics committee approved the experimental protocol. To be accepted into the study, volunteers had to have blood cell counts and clinical chemistry tests within a normal range. Drug screening was performed using qualitative urine tests. An electrocardiogram (ECG) and an EEG were also performed along with a physical examination and a medical history. Subjects were excluded from the study if they showed signs of acute or chronic disease, alcoholism, were using any medication, were doing shift work, or had a history of chronic disease or allergies.

Experimental procedure. A single blind, placebo-controlled and balanced design was used, with placebo and experimental conditions separated by at least 7 days. Subjects slept in a sound-attenuated sleep laboratory between 2300 and 0700 for an adaptation night, wearing electrodes and a rectal thermistor probe. In the morning, the blood was tested again to ensure that the blood cell counts were still in the normal range. If there were abnormal cell counts or clinical signs of developing infection, subjects were rescheduled for testing at a later date. Otherwise, subjects were asked to return to the laboratory at 1200 for lunch and then again at 1630. At 1700, an intravenous catheter was inserted into an antecubital forearm vein, and the subjects were served a light evening meal at 1800. Blood was sampled half-hourly beginning at 1800 and continuing through the night until 1200 the next day. A history of side effects was used to assess the subjective experience of awakening in the morning and hourly thereafter until the subjects left the laboratory. All subjects returned to the laboratory, at least 1 wk later, to go through the same procedure for the other (balanced) condition. Therefore, all subjects were studied under both placebo and endotoxin conditions.

Volunteers wore electrodes for the measurement of EGG; electrooculogram (EOG) and chin surface electromyogram (EMG) were placed according to standardized methods (34). In addition, two chest electrodes were placed to record a one-lead ECG. Rectal temperature was monitored continuously. If they awoke before 1200, they were served breakfast and were required to stay in bed until noon.

Sleep analysis. EEG, EOG, and EMG were recorded on paper and visually scored according to standardized procedures for the classification of stages 1, 2, 3, and 4, and REM sleep (34) by trained scorers blind with respect to the treatment conditions. Composite variables were also computed and are defined in Table 1: deep NREM sleep (stages 3 and 4), movement time, wake after sleep onset, sleep onset latency, REM latency, cycle duration, sleep efficiency, sleep fragmentation (an index of sleep disruption), and REM density.

EEG was amplified and recorded using a Schwarzer type ED24 polygraph (0.53-Hz high-pass and 50-Hz notch filter, calibrated at 50 μV with a 10-Hz sine wave), digitized and sampled with an analog-to-digital converter at a rate of 97.1 Hz, and stored on computer for subsequent spectral analysis (45, 46). The C4-A1 derivation was submitted to a Fast Hartley transformation (4, 45) after artifact-contaminated data had been eliminated and power spectra were computed from the time lights were turned off, immediately after injection, for 8 h, collapsed across stages of sleep, and performed for NREM (stages 1–4) and REM sleep separately. Spectra comprised 50 frequency bins of 0.38-Hz intervals, stepping up from 0 to 48.26 Hz. Aliasing could be expected at frequencies >30 Hz, and these frequencies were excluded from analyses. Power calculated across the delta (0.76–4.18 Hz), theta (4.56–7.98 Hz), alpha (8.36–11.78 Hz), sigma (12.16–14.44 Hz), and beta (14.82–18.62 Hz) frequency bands was analyzed.

Blood sampling and assays. Blood sampled for leukocyte counts was stabilized with Na-EDTA and analyzed with a Coulter Counter ST3 (Coulter, Krefeld, Germany). Blood samples taken for plasma hormone and cytokine assays were stabilized with Na-EDTA (1 mg/ml blood) and Aprotinin (300 kallikrein inhibiting units/ml blood) immediately after drawing, and kept on ice for 5 min before centrifugation for 10 min at 4°C and 2,600 g. Plasma was subsequently frozen to −20°C for later assay. The cytokines IL-6, TNF-α, and soluble TNF receptor (sTNFR) p55 and p75 were determined using enzyme-linked immunoabsorbent assays (Medgenix Diagnostics, Brussels, Belgium). Inter- and intra-assay coefficients of variation were <5% for all assays. Detection limits for IL-6 and TNF-α were 3 pg/ml for soluble TNF receptor p55, it was 50 pg/ml; and for p75, it was 100 pg/ml. IL-1 receptor antagonist (IL-1ra) was also determined using an enzyme-linked immunoabsorbent assay (R&D Systems, Minneapolis, MN). Inter- and intra-assay coefficients of variation were <8%, detection limits were 22 pg/ml. Cortisol was assayed using coated tube RIA methods (ICN Biomedicals, Carson, CA), with a sensitivity of 1.5 ng/ml and intra- and interassay coefficients of variation <7%.

Statistical analyses. Statistical analyses were performed using the software package SPSSPC. One between-subjects factor, i.e., dose, and one within-subjects factor, i.e., time course, were analyzed for difference in data (endotoxin minus placebo). Difference data were used to quantify endotoxin-induced effects after the removal of known circadian variation in several parameters tested. Significant interactions (dose × time course) were followed with paired t-tests comparing endotoxin vs. placebo for each dose at each time point.

In addition to testing for interactions between condition (placebo, endotoxin) and dose (0.2, 0.4, 0.8 ng/kg), statistical tests were performed by collapsing across dose to test the hypotheses that endotoxin would lead to decreased REM
sleep, increased stage 2 sleep, and sleep disruption. The EEG data were Z-transformed (converted to standardized scores) before statistical analysis because these data show large individual differences and distributions tend to be non-Gaussian. The data were normalized for each subject by pooling data across endotoxin and placebo conditions. The alpha level for rejection of the null hypothesis was set to $P < 0.05$. Trends are reported for comparisons where $P < 0.10$.

In summary, the cytokine, neuroendocrine, thermoregulatory, and heart rate changes were dose dependent and demonstrated a progressive increase from the lowest to the highest dose tested. It is important for the interpretation of the effects of endotoxin on sleep, described below, that the lowest dose of endotoxin administered clearly stimulated the release of cytokines and soluble cytokine receptors, but it did not affect the HPA system and rectal temperature.

Nocturnal sleep. Table 1 shows the descriptive statistics and results of repeated-measures ANOVAs for the overall effects of endotoxin on nocturnal sleep. A significant dose by condition interaction effect was found for the amount of deep NREM sleep. This effect was not attributable to within-subject differences at the higher doses, but only to an increased amount of deep NREM sleep after 0.2 ng/kg of endotoxin. At the lowest dose, time spent in deep NREM sleep was increased for every subject.

On the basis of a previous study by Pollmächer et al. (33) on the effects of endotoxin on nocturnal sleep, an increase in stage 2 sleep and a decrease in REM sleep were anticipated. An increase in stage 2 sleep was not seen, but the previously reported global reduction in REM sleep was noted. This tendency was consistent across doses, however, it did not reach significance when tested separately for the three doses. Keeping with the REM suppression, there was an overall endotoxin-induced increase in REM latency that, again, was not significant for any one dose considered alone.

Table 1. Dose-dependent effects of endotoxin on sleep

<table>
<thead>
<tr>
<th>Dose (ng/kg)</th>
<th>Placebo</th>
<th>Endotoxin</th>
<th>Placebo</th>
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<tr>
<td>Total sleep time</td>
<td>574.1 (32.7)</td>
<td>550.9 (48.6)</td>
<td>561.3 (30.8)</td>
<td>562.6 (31.2)</td>
<td>609.1 (45.0)</td>
<td>599.4 (52.6)</td>
<td>NS</td>
<td>NS</td>
<td></td>
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<tr>
<td>Stage 1</td>
<td>64.2 (10.8)</td>
<td>54.8 (8.2)</td>
<td>41.3 (5.2)</td>
<td>46.8 (15.4)</td>
<td>68.5 (9.8)</td>
<td>70.4 (10.5)</td>
<td>NS</td>
<td>NS</td>
<td></td>
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<tr>
<td>Stage 2</td>
<td>317.1 (16.1)</td>
<td>301.2 (38.0)</td>
<td>296.6 (17.0)</td>
<td>328.4 (24.0)</td>
<td>308.8 (24.9)</td>
<td>325.7 (35.7)</td>
<td>NS</td>
<td>NS</td>
<td></td>
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<tr>
<td>Deep NREM (stages 3 + 4)</td>
<td>40.6 (5.8)</td>
<td>54.4 (5.8)*</td>
<td>78.3 (10.0)</td>
<td>65.4 (10.3)</td>
<td>65.2 (10.8)</td>
<td>70.4 (10.5)</td>
<td>NS</td>
<td>NS</td>
<td></td>
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<tr>
<td>REM</td>
<td>147.2 (14.3)</td>
<td>133.7 (6.4)</td>
<td>136.2 (15.4)</td>
<td>119.2 (16.3)</td>
<td>156.6 (25.9)</td>
<td>118.4 (22.0)</td>
<td>F = 5.71†</td>
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<td>Movement time</td>
<td>5.1 (1.1)</td>
<td>7.0 (2.5)</td>
<td>7.8 (1.7)</td>
<td>9.0 (1.9)</td>
<td>10.0 (2.1)</td>
<td>11.0 (1.7)</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Wake after sleep onset</td>
<td>317.1 (16.1)</td>
<td>301.2 (38.0)</td>
<td>296.6 (17.0)</td>
<td>328.4 (24.0)</td>
<td>308.8 (24.9)</td>
<td>325.7 (35.7)</td>
<td>NS</td>
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<tr>
<td>Sleep onset latency</td>
<td>41.4 (6.9)</td>
<td>31.7 (5.1)</td>
<td>30.3 (10.9)</td>
<td>60.8 (36.9)</td>
<td>41.9 (9.6)</td>
<td>105.9 (20.6)†</td>
<td>F = 4.46 NS</td>
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<tr>
<td>REM latency</td>
<td>82.8 (12.1)</td>
<td>94.0 (16.0)</td>
<td>43.5 (11.5)</td>
<td>101.1 (37.0)</td>
<td>58.6 (17.8)</td>
<td>115.5 (51.4)</td>
<td>F = 5.19†</td>
<td>NS</td>
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<tr>
<td>Cycle</td>
<td>100.9 (6.4)</td>
<td>102.4 (3.5)</td>
<td>92.7 (4.9)</td>
<td>106.0 (10.6)</td>
<td>93.5 (5.3)</td>
<td>110.8 (10.4)</td>
<td>F = 3.87†</td>
<td>NS</td>
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<tr>
<td>Sleep efficiency</td>
<td>92.0 (2.0)</td>
<td>96.0 (1.0)</td>
<td>95.0 (2.0)</td>
<td>93.0 (4.0)</td>
<td>93.0 (2.0)</td>
<td>84.0 (4.0)†</td>
<td>NS</td>
<td>F = 3.62†</td>
<td></td>
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<tr>
<td>Sleep fragmentation</td>
<td>105.6 (12.2)</td>
<td>86.2 (9.7)</td>
<td>71.7 (15.3)</td>
<td>107.7 (51.7)</td>
<td>110.4 (2.0)</td>
<td>178.3 (72.7)†</td>
<td>NS</td>
<td>NS</td>
<td></td>
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<tr>
<td>REM density</td>
<td>2.3 (0.2)</td>
<td>2.5 (0.3)</td>
<td>2.2 (0.4)</td>
<td>2.2 (0.4)</td>
<td>2.0 (0.2)</td>
<td>2.1 (0.3)</td>
<td>NS</td>
<td>NS</td>
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Values represent means ± SD in parentheses in minutes. Deep NREM sleep, non-rapid eye movement sleep stages (3 + 4); REM, rapid eye movement; movement time, time spent moving during sleep; wake after sleep onset, amount of time during the sleep period spent in wakefulness; sleep onset latency, time between start of recording and 1st 30-s page scored as stage 2 or greater stage of sleep; REM latency, time between sleep onset and 1st page of REM sleep; cycle duration, time from the 1st page after the end of REM to the start of the next REM period; sleep efficiency, percentage of sleep time spent asleep; sleep fragmentation, time of sleep period spent in wake and/or stage 1; REM density, average number of 3-s miniphasos per 30-s containing REMs; C, condition; D, dose; NS, not significant; df, degrees of freedom. Significance levels: *$P < 0.01$; †$P < 0.05$; ‡$P < 0.10$. 

In the text and Table 1, means ± SD are reported, and in Figs. 1–4, means ± SE are depicted.
Sleep efficiency showed a trend toward a significant dose by condition interaction, due to sleep disruption after the administration of 0.8 ng/kg of endotoxin, reflected also in the significantly increased sleep disruption. However, neither sleep efficiency, wakefulness after sleep onset, nor sleep disruption were significantly affected by the other doses tested. No significant influence of endotoxin administration on other aspects of global nocturnal sleep architecture could be ascertained.

Digitized EEG was analyzed separately for both REM and NREM sleep, and endotoxin and placebo conditions were compared for the seven subjects of the 0.2 ng/kg condition. No significant differences were seen for any of the power spectra during NREM sleep, but trends were evident for delta [F(1,6) = 4.7, P < 0.10], theta [F(1,6) = 4.97, P < 0.10], and alpha [F(1,6) = 4.8, P < 0.10]. No significant differences or trends were found for REM sleep analyses. Because there were no differences between endotoxin and placebo conditions in cycle length (see Table 1), we collapsed all stages and analyzed frequency spectral bands across the night. Delta, theta, and alpha power in the 0.2 ng/kg group were significantly higher following endotoxin than following placebo, 167.8 ± 30.3 vs. 144.8 ± 23.4 µV² [F(1,6) = 14.8; P < 0.01]; 19.5 ± 2.3 vs. 18.0 ± 6.2 µV² [F(1,6) = 6.3; P < 0.05]; 9.5 ± 3.0 vs. 8.9 ± 3 µV² [F(1,6) = 6.2; P < 0.05], respectively.

Time course of nocturnal sleep. The time course of the sleep response to the endotoxin challenge is seen in Fig. 3. Endotoxin at 0.8 ng/kg induced a prominent sleep disruption during the first half of the night, peaking 2 h after the administration of endotoxin. At the same time, there was a reduction in the amount of NREM sleep that showed a rebound in the second half of the night. Whereas 0.4 ng/kg had no major effect on the time course of nocturnal sleep, 0.2 ng/kg reduced the amount of wakefulness plus stage 1 sleep and increased the amount of NREM sleep during the first hours after the administration of endotoxin.

Because the results of visual scoring for the 0.8 and 0.4 ng/kg groups were qualitatively similar, with a
difference in degree only and because there were technical failures during the digital data acquisition of two subjects in the 0.8 ng/kg group and one subject in the 0.4 ng/kg group, data from both of these dose groups were combined (n = 8) for the analysis of endotoxin’s effects on EEG spectra. For the 0.2 ng/kg condition, data from all seven subjects were available. Data from the first 8 h of sleep were collapsed across sleep stages.
and compared between conditions for the combined 0.8 and 0.4 ng/kg doses and for the 0.2 ng/kg dose group. These whole night data were divided into half hourly bins; the first bin synchronized with the time of injection. As seen in Fig. 4, Z-transformed delta power data showed a significant endotoxin-induced elevation across the night in the 0.2 ng/kg group, but no overall change was found for the group that received the higher doses. Analyzed in half-hour bins, delta power increased between 30 and 60 min after the injection of 0.2 ng/kg, and it decreased nonsignificantly at the same time in the higher doses group. The same pattern repeated in the time bin between 120 and 150 min after injection.

DISCUSSION

Classical host response. In the present study, we used a model of bacterial endotoxemia to study the effects of host defense activation on human sleep. Host defense activation resulted in dose-dependent increases in the plasma levels of TNF-α, sTNFR p55, sTNFR p75, IL-1ra, IL-6, cortisol, and in leukocyte counts, heart rate, and body temperature of healthy young men. These elevations are consistent with several earlier reports on the endotoxin-induced human host response (18, 25, 26, 33, 47) with respect to both dose dependence and temporal response kinetics. Consistent with other studies, we found a cascade of events that started by 60 min postendotoxin administration. This cascade was led by an increase in the plasma levels of TNF-α and both sTNFR types, followed by elevations in the plasma levels of IL-6 and cortisol. Plasma levels of IL-1ra, heart rate, and rectal temperature lagged behind by 2–3 h. To our knowledge, this is the first study investigating host response to a dose as low as 0.2 ng/kg of
endotoxin. This very low dose was sufficient to increase leukocyte counts and to induce cytokine release, but it failed to elevate heart rate or to induce a cortisol or febrile response.

In contrast to the endotoxin-induced changes in the systemic levels of cytokines, soluble cytokine receptors, cortisol, heart rate, and rectal temperature that increased monotonically from the lowest to the highest dose administered, the dose dependence of the leukocyte response was more complex. The leukocyte response to endotoxin was similar after 0.2 and 0.8 ng/kg and most prominent after 0.4 ng/kg, suggesting an inverted U-shaped dose-response curve. Comparable results have not been reported before.

Sleep-wake response. CNS function is profoundly influenced by host defense activation, and in the present study, sleep and arousal mechanisms were dose dependently altered by the administration of endotoxin. Consistent with earlier findings (2, 18, 33), endotoxin suppressed REM sleep and increased REM latency. This effect was most prominent after 0.8 ng/kg, although statistical analysis did not substantiate a difference between doses. The REM suppressant effects of host response to endotoxin seen here and in previous human studies (18, 19, 33) may be related to increased IL-6, which was previously shown to reduce REM sleep (40).

The present study confirms that higher doses of endotoxin disrupt sleep (18, 33). After 0.8 ng/kg, the amount of wakefulness during the night more than doubled. Furthermore, there was a prominent decrease in NREM sleep amount during the first half of the night, compensated for by a rebound during the second part. Consequently, NREM sleep amount for the entire night was unchanged. NREM sleep and wakefulness after sleep onset were not significantly altered by the 0.4 ng/kg dose of endotoxin. In contrast to the prominent sleep disruption induced by 0.8 ng/kg of endotoxin, 0.2 ng/kg had a consolidating or enhancing effect on sleep. This was evidenced by a reduced amount of sleep disruption (time spent awake or in stage 1 after the onset of sleep) and a relatively greater amount of deep NREM sleep and delta power. The amount of deep NREM sleep, or slow-wave sleep, and spectral EEG power in the delta frequency band were enhanced within 1–2 h after endotoxin administration.

This is the first study to report that endotoxin increases deep NREM sleep (stages 3 and 4) amount and delta power in humans. Although a previous study by Pollmacher et al. (33) found an increase in NREM sleep amount 4 h after the administration of 0.4 ng/kg of endotoxin (and after host response parameters were past peak levels), the change was due to an increased amount of stage 2 sleep, whereas deep NREM sleep and delta power were not affected. Therefore it seems that in humans, increased NREM sleep amount and intensity during host defense activation occur only when activation is very subtle. One caveat is that although our 0.2 ng/kg subjects were randomly assigned to this condition, the placebo group had low amounts of deep NREM sleep amounts relative to the placebo values of the other groups. However, these deep NREM amounts are in line with placebo data of other studies in which forearm catheters were used for blood collection (14, 32, 38). Hence, it may be that the sleep-enhancing effects of this low dose of endotoxin are limited to individuals with low basal levels of deep NREM sleep, but further investigations are required to answer this question.

The results from the human studies are in contrast with a large body of evidence (reviewed in Ref. 44) that in rodents and rabbits, febrile bacterial or viral illnesses and the administration of pyrogenic amounts of bacterial cell wall components like endotoxin and muramyl peptides consistently increase NREM sleep amount and sleep intensity. Sleep disruption, on the other hand, has been found in these species only when host defense was stimulated to a much greater extent (15, 23, 39) than is feasible to do in human studies. Nonetheless, in all species investigated so far, NREM sleep is enhanced and intensified up to a certain threshold of host defense activation, when the effects of this activation reverse to induce sleep disruption. However, this threshold appears to differ between species and is at the lower end of the activation continuum in humans.

Despite this threshold difference between species, the mechanisms underlying host defense-induced changes in sleep-wake behavior are probably similar. On the basis of the results of the current study and on the results from animal research, it appears that the sleep-promoting factors are to be found among the inflammatory cytokines. Although temperature (30) and the HPA system (41) are involved in sleep-wake regulation, cytokines are more likely to play a central role in the sleep changes reported here. One reason for this assumption is that sleep was enhanced only when cytokine levels were elevated in the absence of changes in temperature and cortisol levels.

Other grounds for assuming that cytokines are primarily involved in these sleep-wake changes come from animal literature demonstrating that increased circulating amounts of endogenous antagonists of TNF-α...
and IL-1, namely sTNF receptors and IL-1ra, suppress deep NREM sleep and delta power. NREM sleep is increased by TNF-α (16, 17), and TNF-α antibodies reverse this effect (43). Moreover, the sleep-enhancing effects of TNF-α function through its p55 (7) and not via its p75 receptor (24). Similarly, with IL-1, its type 1 receptor and antagonist are shown to modulate deep NREM sleep in animals (6, 13, 28, 29), although routes by which both IL-1 and TNF enhance sleep appear to be independent of one another (7). However, a role for circulating IL-1 in endotoxin-induced changes in human sleep-wake behavior is questionable because it has repeatedly been shown that endotoxin does not increase peripheral IL-1β levels (2, 19, 26).

Since the discovery of the NREM sleep-promoting effects of muramyl peptides (22), increased NREM sleep amount and intensity in the course of host response to infection have been thought to indicate an immune-supportive function for NREM sleep. However, as the available evidence regarding the effects of sleep deprivation on host defense is conflicting (reviewed in Ref. 3), this hypothesis remains to be proven. The present results suggest that with respect to a bacterial challenge, a presumed immune-supportive function for NREM sleep may be restricted to conditions of subtle host defense stimulation, for example, during the very early stages of infection.

In conclusion, the present study demonstrates that human sleep-wake behavior is very sensitive to host defense activation, probably via an endotoxin-induced release of inflammatory cytokines. These cytokines promote deep NREM sleep amount and intensity if the activation is subtle or leads to considerably disrupted sleep when a stronger activation occurs.

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