Sleep during Experimental Trypanosomiasis in Rabbits (43694)

LINDA A. TOTH,*‡[1] ELIZABETH A. TOLLEY,† ROY BROADY,‡ BARBARA BLAKELY,*[2] AND JAMES M. KRUEGER‡

Departments of Comparative Medicine, Biostatistics and Epidemiology,† and Physiology and Biophysics,‡ University of Tennessee, Memphis, Tennessee 38163*

Abstract. ***Trypanosoma brucei* subspecies cause the human condition known as "sleeping sickness." In rabbits, these organisms induce a chronic and ultimately fatal disease characterized by periodic parasitemia. To characterize sleep alterations during a chronic infectious condition and to determine how immune stimulation of the host, as reflected by cyclic parasitemia, is related to altered somnolence, we monitored sleep and other clinical indices in rabbits inoculated subcutaneously with *Trypanosoma brucei brucei*. Within four days, infected rabbits developed fever, reduced food intake, and other signs of infectious illness concurrent with the onset of parasitemia were evident. The initial febrile episodes were transient, recurring in temporal correlation with parasitemia. Time spent in slow-wave sleep and delta-wave amplitude during slow-wave sleep increased significantly in association with the onset of febrile episodes, despite an overall trend toward decreases in these parameters. Because each episode of parasitemia presents an immune stimulus to the infected host, the periodic enhancement of sleep observed in this model is consistent with the hypothesis that immune stimulation is correlated with increased somnolence. The data further indicate that sleep alterations occur not only during acute infections, as previously reported, but during chronic infections as well.** [P.S.E.B.M. 1994, Vol 205]

Fatigue and an increased desire to sleep are presenting signs for many infectious conditions. Acute bacterial, fungal, and viral infections induce altered sleep patterns in rabbits (1–3). These alterations are characterized by an initial phase of increased sleep that persists for up to 20 hr after inoculation and a subsequent phase of reduced sleep. The precise temporal patterns depend on the infectious agent used and the route of administration (1, 2). Endogenous somnogenic cytokines and other immune modulators are postulated to mediate sleep alterations during infectious disease (4). However, studies of acute clinical syndromes may not necessarily reflect conditions characterized by a prolonged clinical course. The immunologic and pathologic consequences of microbial infections change as the disease becomes chronic. Similarly, sleep perturbations during chronic infectious conditions may persist or, alternatively, may gradually abate, as suggested by the tolerance demonstrated in rabbits repeatedly inoculated with influenza virus and other somnogens (3, 5).

In humans, some *Trypanosoma brucei* subspecies cause a chronic clinical syndrome commonly called "sleeping sickness." Descriptions of this condition refer to lassitude, tiredness, somnolence, insomnia, and coma (6), although a recent study reported that hypersomnia was not characteristic of the disease (7). Rabbits inoculated with *Trypanosoma brucei brucei* (*Tbb*) develop a similar chronic and ultimately fatal wasting disease of up to eight weeks duration (8). *Tbb* infections offer a useful model for evaluating the relationship between microbially-induced sleep alterations and endogenous immune modulators. Trypanosomes undergo antigenic variation in the host (9), proliferating as new variants emerge and declining after stimulation of a host immune response. Elevated levels of endogenous immune modulators such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) probably con-

[1] To whom requests for reprints should be addressed at Animal Resource Center, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38105.
[2] Present address: Animal Resource Center, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38105.

Received July 14, 1993. [P.S.E.B.M. 1994, Vol 205]
Accepted October 27, 1993.

0037-9727/94/2052-0174$10.50/0

tribute to the host's defense during trypanosomiasis and may secondarily mediate the somnogenic manifestations of the disease (4). We examined sleep patterns during trypanosomiasis in rabbits to determine how sleep is altered during a chronic infectious condition and how immune stimulation of the host, as reflected by cyclic parasitemia, is related to altered somnolence.

Materials and Methods

Adult male New Zealand white rabbits (*Pasteurella multocida*–free) (Myrtle's Rabbitry, Thompson Station, TN) weighing 4–5 kg were surgically implanted with electroencephalographic (EEG) recording electrodes and brain thermistors as previously described (10) and were allowed to recover for several weeks prior to use. Rabbits were housed individually on a 12:12 hr light-dark schedule in a temperature-controlled room (21 ± 2°C). Prior to testing, rabbits were allowed to adapt overnight to recording chambers maintained under the same conditions. Sleep, food intake, and other parameters were then monitored for 24 hr without experimental treatment. The next day at approximately 8:00 AM, some rabbits (n = 8) were inoculated subcutaneously with approximately 4×10^6 *Tbb* organisms suspended in sterile pyrogen-free phosphate-buffered saline. Other rabbits (n = 4) were similarly inoculated with an equivalent volume of an autoclaved *Tbb* suspension. All rabbits were then monitored for an additional 15–23 days. Rectal temperatures were measured daily at approximately 8:00 AM. Food intake was measured daily by weighing the feed container; no attempt was made to correct for spillage. Blood samples were collected at approximately 8:00 AM on Mondays, Wednesdays, and Fridays, and animals were weighed weekly. Rabbits could move freely in their cages throughout the experiment, and had continuous access to food and water. Each rabbit was inoculated only once and was euthanized with intravenous T-61 euthanasia solution (Hoechst-Roussel, Somerville, NJ) at the end of the recording period.

EEG and brain temperature data were recorded via a rotary commutator (Plastics One, Roanoke, VA). Animal movement was monitored via an acceleration transducer (Grass Instruments, Quincy, MA) connected to the EEG cable. Delta (0.5–4.0 Hz) wave components of the EEG were quantified using band-pass filters (Buxco Electronics, Sharon, CT) and analog-to-digital conversion. The analog signals of EEG activity, the filtered rectified delta wave component, animal movement and brain temperature were displayed on a polygraph (Grass Instruments, Quincy, MA). Average EEG delta wave amplitudes (DWA) were calculated for each 1-min interval of the recording period and stored in digital form on computer.

Vigilance states were determined based on DWA, movement, and brain temperature (10) using computer-assisted scoring. Each animal's EEG tracing, filtered and rectified EEG delta wave signal, and movement recording for the first 6 hr of the baseline period were visually examined to determine a threshold DWA associated with slow-wave sleep (SWS). This value was used to determine vigilance states for each animal during each 1-min interval throughout the recording period. An animal was considered to be in SWS whenever the average DWA during any 1-min interval exceeded the SWS threshold amplitude in the absence of movement. When not in SWS, the animal was either awake or in rapid-eye-movement sleep (REMS). REMS was identified by visually inspecting the polygraph record for a low-voltage EEG tracing associated with a rise in brain temperature and sporadic movements (occasional twitching) (10). Sleep parameters were summarized across 12-hr intervals corresponding to daily light and dark periods. The percentage of time spent in SWS and REMS, the average DWA during SWS, and the average lengths of SWS bouts were calculated for each animal. In addition, a sleep quality score (SQS) was calculated for each 12-hr period of the experiment as the product of the percent time in SWS and the DWA during SWS (expressed as a percentage of baseline values) divided by 100, thus reflecting both the duration and the intensity or depth of sleep (11).

Blood samples (1–3 ml) were collected from the central artery of the ear and immediately transferred into vacuum tubes containing EDTA. Total white blood cell (WBC) counts were measured using a hemocytometer. Differential WBC counts were made by classifying 100 WBCs on Wright-stained blood smears. Numbers of trypanosomes and nucleated red blood cells (nRBC) per 100 WBC were counted using the same smears. Trypanosome counts were converted to the number of organisms present per 100 μl of blood. Hematocrits were measured as packed cell volumes.

Data were analyzed by using two-way analysis of variance for repeated measures (12). Fisher's least-significant-difference test was used for a priori comparisons of individual means (13). Regression analyses were performed using the general linear models procedure (12). Student's t-test was used to compare differences in body weights. A significance level of $P < 0.05$ was used.

Results

Rabbits remained physiologically normal for up to four days after inoculation with trypanosomes, as reflected by body temperature, food intake and hematologic data (Fig. 1 and 2). On Day 6, infected rabbits became febrile (Fig. 1). The fevers abated after four days but reappeared on approximately Day 12. The

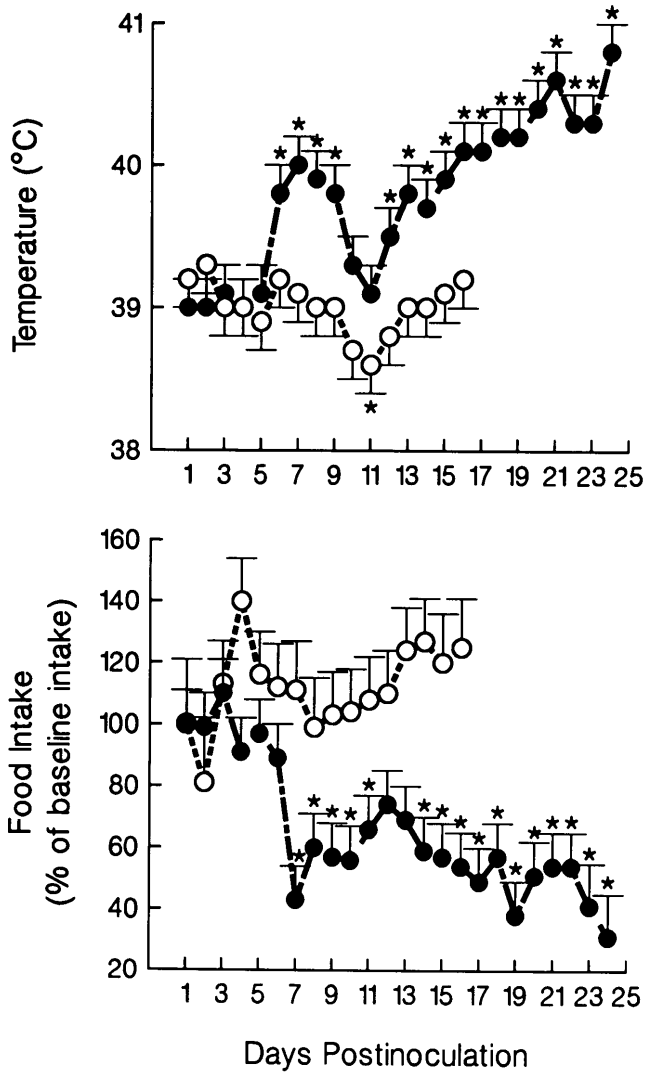

Figure 1. Rectal temperature and food intake in rabbits inoculated with *Trypanosoma brucei brucei* (filled circles; $n = 8$) or with an equivalent inoculum of heat-killed organisms (open circles; $n = 4$). Food intake is expressed as a percentage of preinoculation values (control group, 91 ± 19 g; experimental group, 140 ± 16 g). Data points represent the mean ± SEM. *$P < 0.05$ relative to the baseline value for that group.

onset of fever was temporally correlated with the presence of trypanosomes in blood (Fig. 2). Fever and parasitemia preceded a sustained decrease in food intake (Fig. 1) that resulted in significant weight loss (4.75 ± 0.10 kg on Day 1 vs 4.18 ± 0.12 kg on Day 20; $P < 0.025$). Anemia, leukopenia (primarily lymphopenia; data not shown), and elevated nRBC counts also developed subsequent to parasitemia (Fig. 2). No signs of neurologic disease were observed in any trypanosome-inoculated rabbit. One infected rabbit was euthanized on Day 15 because of severe dehydration and diarrhea.

Rabbits inoculated with viable trypanosomes demonstrated a significant increase in SQS during the daylight hours on Day 6 postinoculation (Fig. 3), coincident with parasitemia and the onset of fever. The in-

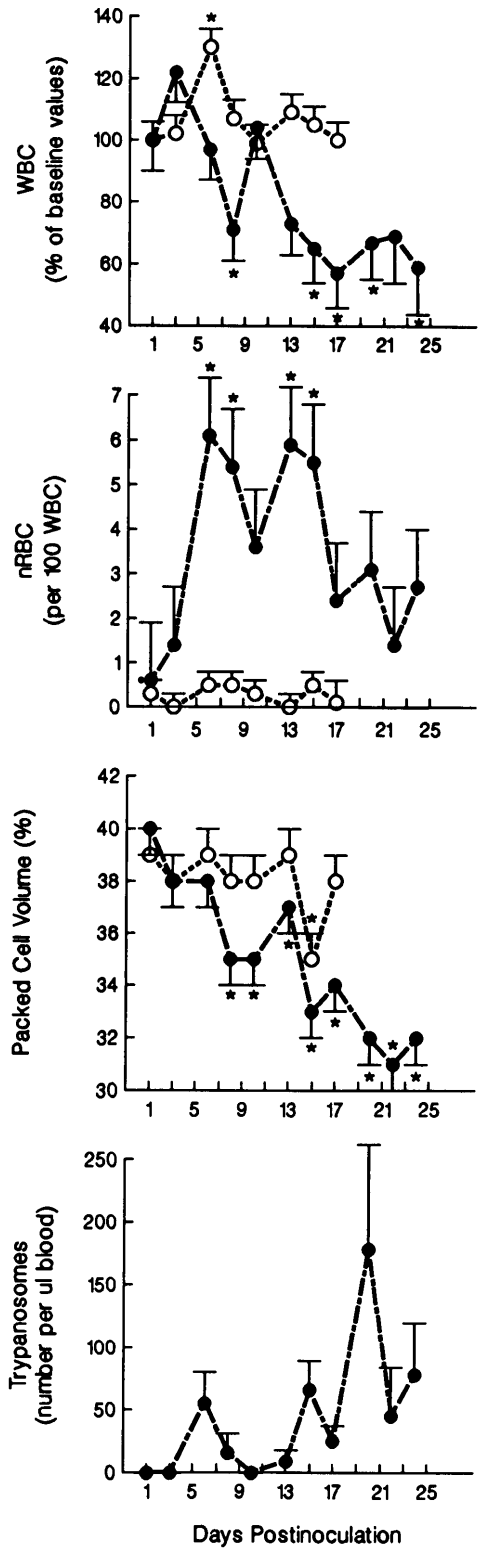

Figure 2. Hematologic parameters of rabbits inoculated with *Trypanosoma brucei brucei* (filled circles; $n = 8$) or with an equivalent inoculum of heat-killed organisms (open circles; $n = 4$). WBC counts are expressed as a percentage of preinoculation values (control group, 7416 ± 410 cells/μl; experimental group, 9861 ± 989 cells/μl). Data points represent the mean ± SEM. *$P < 0.05$ relative to the initial value obtained for that group.

creased SQS reflected moderate increases in both the amount of SWS and in DWA during SWS. However, a significant overall downward trend in SWS time and DWA developed during both the light (SWS, slope = -0.25, $P < 0.02$; DWA, slope = -1.05, $P < 0.0001$) and dark periods (SWS, slope = -0.36, $P < 0.0008$; DWA, slope = -0.88, $P < 0.0001$). SWS bout length was also significantly reduced during the dark periods in infected rabbits (data not shown). Theta (4.0–8.0 Hz) wave amplitudes during wakefulness were not significantly reduced in infected rabbits (data not shown), indicating that the observed decreases in SWS and DWA could not be attributed to a general reduction in the overall amplitude of the EEG.

In contrast to infected rabbits, rabbits inoculated with heat-killed trypanosomes did not demonstrate consistent deviations in REMS or DWA during the postinoculation period (Fig. 3), but did show a slight but significant time-dependent decrease in SWS during the dark period (slope = -0.42, $P < 0.0001$), resulting in a decrease in the derived SQS (slope = -0.46, $P < 0.0002$). The changes in SWS time did not differ significantly between experimental and control groups, but DWA values were different during both light and dark phases of the circadian cycle ($P < 0.0001$). Secondary to the changes in DWA and SWS time, SQS also decreased significantly with time in trypanosome-treated rabbits (light, slope = -0.71, $P < 0.0001$; dark, slope = -0.60, $P < 0.0001$). The amount of time in REMS decreased at the onset of parasitemia and remained reduced for the remainder of the recording period, resulting in a significant downward trend during both the light (slope = -0.107, $P < 0.0001$) and the dark periods (slope = -0.046, $P < 0.003$) (Fig. 3). Time in REMS did not change significantly in the control group.

The period between the initial and secondary febrile episodes varied considerably among rabbits. To control for this inconsistency, sleep indices measured near the onset of febrile episode were compared to

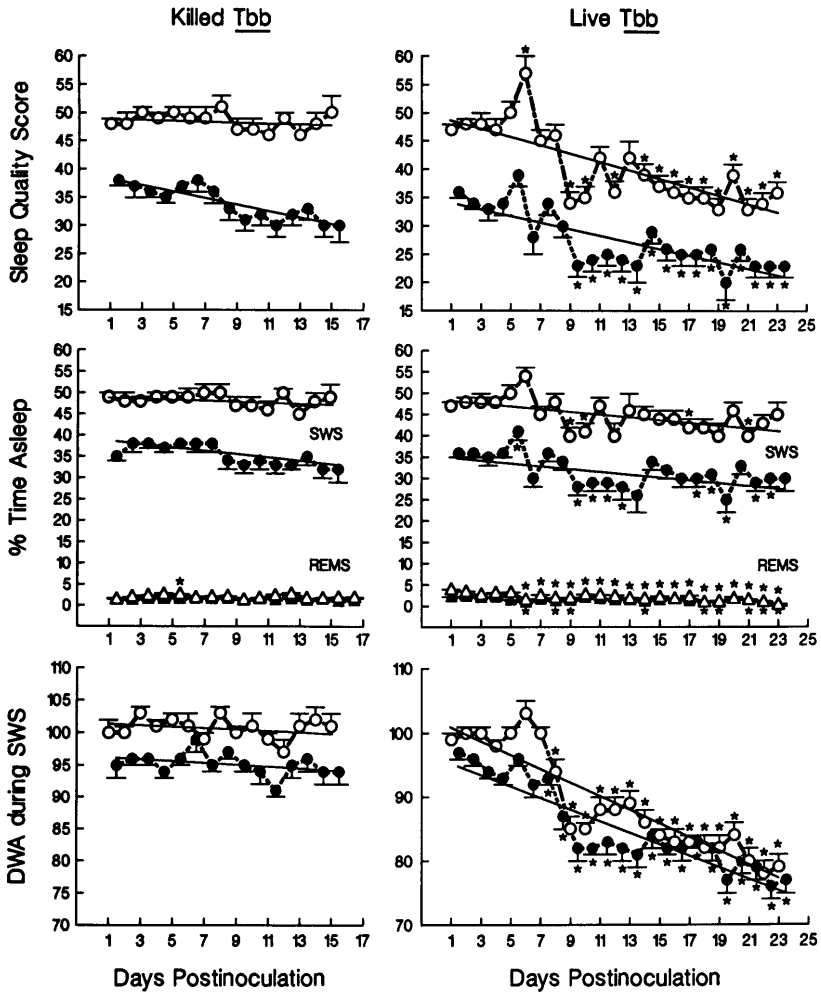

Figure 3. Sleep parameters of rabbits inoculated with *Trypanosoma brucei brucei* (right panels; $n = 8$) or with an equivalent inoculum of heat-killed organisms (left panels; $n = 4$). Data were obtained during 12-hr light periods (open circles) and 12-hr dark periods (filled circles). Solid lines show linear regressions. Sleep quality score is calculated as (% time in SWS) × (DWA during SWS) ÷ 100. Data points represent the mean ± SEM. *$P < 0.05$ relative to the initial value obtained for that group.

those obtained during the immediately preceding afebrile period (Fig. 4). Because rectal temperatures were measured only once daily, the precise time of fever onset could not be specifically determined. Therefore, sleep data collected during the 24-hr periods before and after detection of each febrile episode were summarized for comparison with the preceding afebrile period. Values for the control group were determined during comparable postinoculation intervals. All evaluation periods fell within the following intervals: prefebrile period (39.0 ± 0.1°C), Days 2–3 postinoculation; initial febrile episode (40.2 ± 0.1°C), Days 5–8; interim afebrile period (39.1 ± 0.1°C), Days 8–13; second febrile episode (40.4 ± 0.1°C), Days 11–15. Despite the general tendency toward decreasing sleep time, SQS during the day was significantly higher during febrile than during preceding afebrile periods. This effect was primarily attributable to increased SWS time during light periods in febrile animals. DWA during SWS also increased significantly during the second febrile interval.

Discussion

Trypanosomiasis induced significant sleep alterations in rabbits. A general downward trend in SWS time and DWA during SWS occurred in infected animals, and the circadian variation in DWA values diminished gradually with time. These findings are consistent with a recent report that human trypanosomiasis is characterized by a disruption of the circadian pattern of sleep and wakefulness but is not associated with hypersomnia (7). However, despite the chronic trend toward reduced sleep, SWS time and DWA during SWS increased significantly in association with the onset of febrile episodes. With the initial development of clinical illness, fever, parasitemia, and sleep enhancement occurred in close temporal association. The second febrile episode was also associated with increased sleep, although the precise relationship to parasitemia was less clear because blood samples were not obtained on a daily basis. In chronic trypanosomiasis, cycles of parasitemia mark the emergence of new trypanosomal variants and renewed host immune stimulation (14). Recurrent fever is likely to reflect these cycles. Concurrent increases in sleep are consistent with previous suggestions that the immune response contributes to sleep enhancement during infection (15). Thus, the chronicity of the disease state is characterized by a progressive reduction in sleep, but acute clinical exacerbations are associated with enhanced sleep similar to that observed during acute microbial disease induced by other microorganisms.

The sleep alterations associated with infection and the immune response may reflect altered production

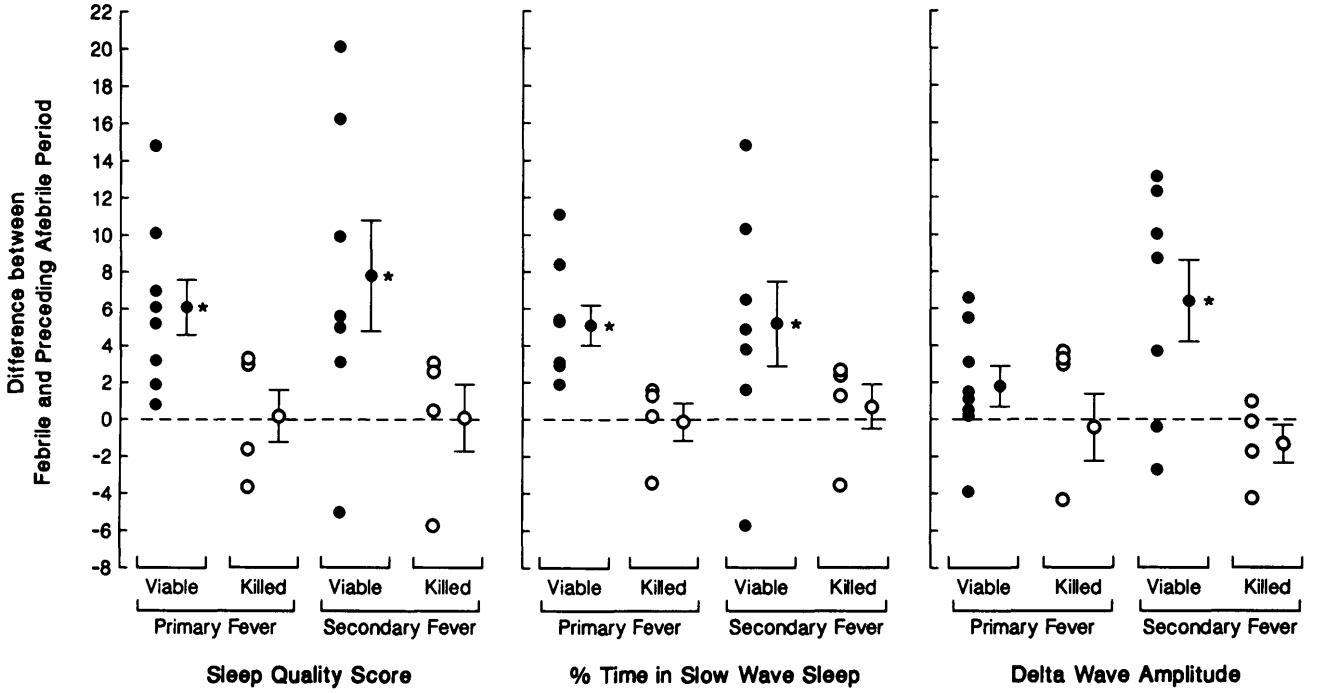

Figure 4. Sleep during febrile episodes in rabbits inoculated with viable (filled circles; $n = 8$) or heat-killed (open circles; $n = 4$) *Trypanosoma brucei brucei* organisms. Sleep parameters were evaluated during the first and second febrile episodes of the infected rabbits and during the two preceding afebrile periods. Data shown were collected during the 12-hr light phase of the circadian cycle; significant differences between febrile and afebrile periods were not observed during the dark phase. Parameters evaluated were sleep quality score ([% time in SWS] × [DWA during SWS] ÷ 100), % of time spent in SWS, and the EEG delta wave amplitude during SWS. Data points represent the differences between febrile-period and preceding afebrile-period values in individual rabbits. Mean ± SEM is shown at the right of each data column. *$P < 0.05$ relative to the preceding afebrile period.

and release of endogenous somnogenic immune modulators such as IL-1, interferon, and TNF, which are known to be somnogenic in rabbits and other species (16–19). TNF activity is known to be elevated in rabbits with trypanosomiasis (20). In mice, parasitemia is temporally associated with increased release of IL-1 from peritoneal macrophages (21) and with a transient increase in serum interferon α/β (22). In late-stage human sleeping sickness, the cerebrospinal fluid has normal IL-1 concentrations but elevated prostaglandin D_2 levels and other clinicopathologic abnormalities (23). In that study, IL-1 activity was measured using a thymocyte proliferation assay. Thus, cytokines known to inhibit IL-1 (*e.g.*, IL-4, IL-10, TGFβ, IL-1 receptor antagonist) could have masked increased IL-1 levels. Nonetheless, the study suggests that somnogenic mediators other than IL-1 could contribute to altered vigilance during human trypanosomiasis. Prostaglandin D_2, for example, increases sleep in monkeys, rats, and cats (24–26), although it is not somnogenic in rabbits (27).

The increased sleep that occurred during trypanosomiasis was temporally associated with the onset of fever. The somnogenic cytokines discussed above are well-known endogenous pyrogens (28) and are likely to cause both fever and enhanced sleep during parasitemia. However, several lines of evidence indirectly suggest that the sleep responses are not dependent on the development of fever. First, trypanosome-infected rabbits, as well as rabbits inoculated with bacteria or fungi, develop fevers that persist longer than the period of enhanced sleep (1, 2). Second, antipyretic drugs block IL-1-induced fevers in rabbits but do not attenuate sleep responses (29). Third, some substances, such as corticotropin releasing factor and prostaglandin E_2, increase body temperature but inhibit sleep (27, 30). Finally, muramyl dipeptide, a somnogenic constituent of bacterial cell walls, induces sleep and fever in rabbits injected during the day but causes only sleep enhancement if injected at night (31). Thus, the mechanisms that mediate sleep enhancement and fever during infectious conditions can clearly be dissociated.

The data reported in this study were collected before trypanosome-induced histopathologic changes would be expected to develop in the CNS of rabbits (32). Prolonged trypanosomiasis, however, is associated with meningoencephalitis in many species, including humans (8, 33–35). In fact, the descriptor "sleeping sickness" may in part reflect observations that patients become semicomatose late in the disease, secondary to nervous system involvement (6). In such cases, the role of functional CNS damage must be distinguished from immune influences in the generation of altered somnolence. During at least the early stages of trypanosomal meningoencephalitis in humans, abnormalities in cortical evoked potentials are not prominent, indicating the absence of overt functional damage (36). These patients demonstrate an abnormal circadian organization of sleep in the absence of hypersomnia (7). Even in late-stage sleeping sickness patients, neuronal damage and inflammation in the CNS parenchyma are minimal despite progressive meningitis, and inflammatory reactions are largely restricted to perivascular cuffs (37). However, peripheral macrophages, lymphocytes and other inflammatory cells are recruited to the brain during even mild CNS inflammation, and phagocytic cells endogenous to the CNS can produce IL-1 and other cytokines, especially in response to CNS injury (38–40). Mice with meningoencephalitis secondary to *Tbb* inoculation, for example, demonstrate astrocyte activation that is correlated with increased mRNA for IL-1 and TNFα in brain homogenates (41, 42). This array of cytokines, or their central distribution, may differ from those that develop during peripheral infections, and may therefore induce different patterns of sleep alteration.

The mechanisms responsible for the progressive decrease in DWA during SWS and in the amount of SWS in general are unknown. Like the trypanosome-infected rabbits, the noninfected control group demonstrated some lack of temporal stability in SWS time, the derived SQS values, and in rectal temperatures. Because these data were collected from six separate pairs of animals evaluated over an eight-month period, aberrant variations in the recording equipment or in the housing conditions are unlikely to account for these effects. Because animals used in this study were evaluated over a much longer interval than is common in most sleep studies, we suspect that these effects may be attributable at least in part to long-term adaptation of the animals to the recording conditions, as reported by others (43). In infected animals, the marked decrease in DWA during SWS may reflect reduced sleep intensity, disturbed sleep or more frequent arousals. Such effects could be caused by chronic production of one or more mediators that reduce SWS and DWA in rabbits, including corticotropin releasing factor (30), prostaglandin E_2 (27), the IL-1 receptor antagonist (44) and α-melanocyte stimulating hormone (45). The sleep reduction may be related to the worsening clinical condition of the animals, as suggested by a recent report that reduced sleep during various infections is associated with more severe clinical signs and an increased probability of death (46). However, sleep indices were positively correlated with disease severity in a recent study of eight humans with clinical trypanosomal meningoencephalitis (7). To the extent that immune mechanisms contribute to enhanced somnolence during infectious conditions, the immune suppression that occurs in advanced *Tbb* infections (14, 21) may reduce sleep. For

example, when rabbits are pretreated with immunosuppressive doses of cortisone, the somnogenic manifestations of bacterial infections are markedly attenuated (47). Furthermore, in humans infected with the human immunodeficiency virus (HIV), sleep is greatly disrupted after overt disease develops secondary to immune impairment (48), although healthy HIV-seropositive patients show increased Stage IV sleep (the human equivalent of SWS) (49).

Trypanosome-infected rabbits demonstrate many facets of the acute-phase response, including fever and elevated reactants such as fibrinogen and C-reactive protein (32, 50, 51). Recent reviews have suggested that sleep is a behavioral component of the acute-phase response, providing a survival advantage during microbial infections (15, 52). However, the functions of increased somnolence during microbial disease remain conjectural, as do other facets of the acute-phase response. Sleep could contribute by several means to the maintenance of physiologic homeostasis during disease (53). Infectious conditions are frequently associated both with fever, which is metabolically demanding, and with anorexia, which contributes to a caloric deficit. By reducing motor activity and the basal metabolic rate, sleep could help to conserve energy. In addition, the ethological tendency of animals to sleep in protected locations would reduce the possibility of encountering predators while weakened by illness. Finally, recent observations suggest that sleep promotes immune efficacy under some conditions (54–56), and that sleep enhancement is related to a favorable prognosis (46). Taken together, these observations indicate that sleep may confer a survival benefit during both acute infections and during chronic infectious diseases such as trypanosomiasis.

The authors thank Dr. John Swindle for supplying trypanosomes and methods for their propagation, Mr. Larry Counce and Ms. Donna Maxwell for technical assistance, and Ms. Sharon Naron, Dr. Ferenc Obal, and Dr. Levente Kapas for critical review of this manuscript. This work was supported by NIH Grants NS-26429, NS-25378, and NS-27250.

1. Toth LA, Krueger JM. Alterations in sleep during *Staphylococcus aureus* infection in rabbits. Infect Immun **56**:1785–1791, 1988.
2. Toth LA, Krueger JM. Effects of microbial challenge on sleep in rabbits. FASEB J **3**:2062–2066, 1989.
3. Kimura-Takeuchi M, Majde JA, Toth LA, Krueger JM. Influenza virus–induced changes in rabbit sleep and acute phase responses. Am J Physiol **263**:R1115–R1121, 1992.
4. Toth LA, Krueger JM. Somnogenic, pyrogenic and hematologic effects of experimental pasteurellosis in rabbits. Am J Physiol **258**:R536–R542, 1990.
5. Kimura-Takeuchi M, Majde JA, Toth LA, Krueger JM. The role of double-stranded RNA in induction of the acute-phase response in an abortive influenza virus infection model. J Infect Dis **166**:1266–1275, 1992.
6. Apted FIC. Clinical manifestations and diagnosis of sleeping sickness. In: Mulligan HW, Ed. The African Trypanosomiasis. London: George Allen and Unwin Ltd., pp661–683, 1970.
7. Buguet A, Bert J, Tapie P, Tabaraud F, Doua F, Lonsdorfer J, Bogui P, Dumas M. Sleep-wake cycle in human African trypanosomiasis. J Clin Neurophysiol **10**:190–196, 1993.
8. Losos GJ, Ikede BO. Review of pathology of diseases in domestic and laboratory animals caused by *Trypanosoma congolense, T. vivax, T. brucei, T. rhodiense* and *T. gambiense*. Vet Path **9**:1–71, 1972.
9. Barry JD, Turner CMR. The dynamics of antigenic variation and growth of African trypanosomes. Parasitol Today **7**:207–211, 1991.
10. Krueger JM, Davenne D, Walter J, Shoham S, Kubillus SL, Rosenthal RS, Martin SA, Biemann K. Bacterial peptidoglycans as modulators of sleep. II. Effects of muramyl peptides on the structure of rabbit sleep. Brain Res **403**:258–266, 1987.
11. Borbely AA. A two process model of sleep regulation. Human Neurobiol **1**:195–204, 1982.
12. The GLM procedure. SAS/STAT User's Guide Version 6 (4th ed). Cary: SAS Institute, pp891–996, 1989.
13. Sokal RR, Rohlf FJ. Biometry: The Principles and Practice of Statistics in Biological Research (2nd ed). New York: W. H. Freeman, pp232–242, 1981.
14. Mansfield JM. Immunology and immunopathology of African trypanosomiasis. In: Mansfield JM, Ed. Parasitic Diseases. New York: Marcel Dekker, pp167–226, 1981.
15. Toth LA, Krueger JM. Infectious disease, cytokines, and sleep. In: Mancia M, Marini G, Eds. The Diencephalon and Sleep. New York: Raven Press, pp331–341, 1990.
16. Smedley H, Katrak M, Sikora K, Wheeler T. Neurological effects of human recombinant interferon. Brit Med J **286**:262–265, 1983.
17. Krueger JM, Dinarello CA, Shoham S, Davenne D, Walter J, Kubillus S. Interferon alpha-2 enhances slow-wave sleep in rabbits. Int J Immunopharmac **9**:23–30, 1987.
18. Shoham S, Davenne D, Cady AB, Dinarello CA, Krueger JM. Recombinant tumor necrosis factor and interleukin-1 enhance slow-wave sleep. Am J Physiol **253**:R142–R149, 1987.
19. Desarro GB, Masuda Y, Ascioti C, Audino MG, Nistico G. Behavioural and ECoG spectrum changes induced by intracerebral infusion of interferons and interleukin-2 in rats are antagonized by naloxone. Neuropharmacol **29**:167–179, 1990.
20. Rouzer CA, Cerami A. Hypertriglyceridemia associated with *Trypanosoma brucei brucei* infection in rabbits: Role of defective triglyceride removal. Molec Biochem Parasitol **2**:31–38, 1980.
21. Askonas BA, Bancroft GJ. Interaction of African trypanosomes with the immune system. Phil Trans R Soc Lond **307**:41–50, 1984.
22. Bancroft GJ, Sutton CJ, Morris AG, Askonas BA. Production of interferons during experimental African trypanosomiasis. Clin Exp Immunol **52**:135–143, 1983.
23. Pentreath VW, Rees K, Owolabi OA, Philip KA, Doua F. The somnogenic T lymphocyte suppressor prostaglandin D_2 is selectively elevated in cerebrospinal fluid of advanced sleeping sickness patients. Trans R Soc Trop Med Hyg **84**:795–799, 1990.
24. Laychock SG, Johnson DN, Harris LS. PGD_2 effects on rodent behavior and EEG patterns in cats. Pharmacol Biochem Behav **12**:747–754, 1980.
25. Ueno R, Honda K, Inoue S, Hayaishi O. Prostaglandin D_2, a cerebral sleep-inducing substance in rats. Proc Natl Acad Sci USA **80**:1735–1737, 1983.
26. Onoe H, Ueno R, Fujita I, Nishino H, Oomura Y, Hayaishi O. Prostaglandin D_2, a cerebral sleep-inducing substance in monkeys. Proc Natl Acad Sci USA **85**:4082–4086, 1988.
27. Krueger JM, Kapas L, Opp MR, Obal F. Prostaglandins E_2 and

D_2 have little effect on rabbit sleep. Physiol Behav **51**:481–485, 1992.
28. Kluger MJ. Fever: Role of pyrogens and cryogens. Physiol Rev **71**:93–127, 1991.
29. Krueger JM, Walter J, Dinarello CA, Wolff SM, Chedid L. Sleep-promoting effects of endogenous pyrogen (interleukin-1). Am J Physiol **246**:R994–R999, 1984.
30. Opp M, Obal F, Krueger JM. CRF attenuates interleukin-1 induced sleep and fever in rabbits. Am J Physiol **257**:R528–R535, 1989.
31. Shoham S, Krueger JM. Muramyl dipeptide-induced sleep and fever: Effects of ambient temperature and time of injections. Am J Physiol **255**:R157–R165, 1988.
32. January BE, Toth LA, Parker RF. Pathophysiologic correlates of experimental trypanosomiasis in rabbits. Lab Anim Sci **41**:585–589, 1991.
33. Greenwood BM, Whittle HC. The pathogenesis of sleeping sickness. Trans R Soc Trop Med Hyg **74**:716–725, 1980.
34. Poltera AA. Immunopathological and chemotherapeutic studies in experimental trypanosomiasis with special reference to the heart and brain. Trans R Soc Trop Med Hyg **74**:706–715, 1980.
35. Schmidt H. The pathogenesis of trypanosomiasis of the CNS: Studies on parasitological and neurohistological findings in trypanosoma rhodesiense infected vervet monkeys. Virchows Arch **399**:333–343, 1983.
36. Tabaraud F, Hugon J, Buguet A, Lonsdorfer A, Gati R, Doua F, Dumas M. Study of evoked potentials in human African trypanosomiasis. J Trop Med Hyg **95**:246–252, 1992.
37. Pentreath VW. Neurobiology of sleeping sickness. Parasitol Today **5**:215–218, 1989.
38. Fontana A, Kristensen F, Dubs R, Gemsa D, Weber E. Production of prostaglandin E and an interleukin 1-like factor by cultured astrocytes and C6 glioma cells. J Immunol **129**:2413–2419, 1982.
39. Nieto-Sampedro M, Berman MA. Interleukin-1-like activity in rat brain: Sources, targets, and effects of injury. J Neurosci Res **17**:214–219, 1987.
40. Woodroofe MN, Sarna GS, Wadhwa M, Hayes GM, Loughlin AJ, Tinker A, Cuzner ML. Detection of interleukin-1 and interleukin-6 in adult rat brain, following mechanical injury, by *in vivo* microdialysis: Evidence of a role for microglia in cytokine production. J Neuroimmunol **33**:227–236, 1991.
41. Hunter CA, Gow JW, Kennedy PGE, Jennings FW, Murray M. Immunopathology of experimental African sleeping sickness: Detection of cytokine mRNA in the brains of *Trypanosoma brucei brucei*–infected mice. Infect Immun **59**:4636–4640, 1991.
42. Hunter CA, Jennings FW, Kennedy PGE, Murray M. Astrocyte activation correlates with cytokine production in central nervous system of *Trypanosoma brucei brucei*–infected mice. Lab Invest **67**:635–642, 1992.
43. Sinton CM, Valatax JL, Jouvet M. Sleep times and adaptation effects in the mouse. In: WP Koella, Ed. Sleep 1982. Basel: Karger, pp229–231, 1983.
44. Opp MR, Krueger JM. Interleukin-1-receptor antagonist blocks interleukin-1-induced sleep and fever. Am J Physiol **260**:R453–R457, 1991.
45. Opp MR, Obal F, Krueger JM. Effects of α-MSH on sleep, behavior, and brain temperature: Interactions with IL-1. Am J Physiol **255**:R914–R922, 1988.
46. Toth LA, Tolley EA, Krueger JM. Sleep as a prognostic indicator during infectious disease in rabbits. Proc Soc Exp Biol Med **203**:179–192, 1993.
47. Toth LA, Gardiner TW, Krueger JM. Effects of cortisone on sleep in normal and bacterially-infected rabbits. Am J Physiol **263**:R1339–R1346, 1992.
48. Kubicki ST, Henkes H, Terstegge K, Ruf B. AIDS related sleep disturbances—A preliminary report. In: Kubicki ST, Henkes H, Bienzle U, Pohle HD, Eds. HIV and the Nervous System. New York: Gustav Fischer, pp97–105, 1988.
49. Norman SE, Chediak HD, Kiel M, Cohn MA. Sleep disturbances in HIV-infected homosexual men. AIDS **4**:775–781, 1990.
50. Thomasson DL, Mansfield JM, Doyle RJ, Wallace JH. C-reactive protein levels in experimental African trypanosomiasis. J Parasitol **59**:738–739, 1973.
51. Boreham PFL, Facer CA. Plasma fibrinogen in rabbits infected with *T. (T.) brucei*. Trans R Soc Trop Med Hyg **68**:153, 1974.
52. Krueger JM, Majde JA. Sleep as a host defense: Its regulation by microbial products and cytokines. Clin Immunol Immunopathol **57**:188–199, 1990.
53. Hart BL. Biological basis of the behavior of sick animals. Neurosci Biobehav Rev **12**:123–137, 1988.
54. Brown R, Pang G, Husband AJ, King MG. Suppression of immunity to influenza virus infection in the respiratory tract following sleep disturbance. Reg Immunol **2**:321–325, 1989.
55. Brown R, Price RJ, King MG, Husband AJ. Interleukin-1 β and muramyl dipeptide can prevent decreased antibody response associated with sleep deprivation. Brain Behav Immun **3**:320–330, 1989.
56. Everson CA. Sustained sleep deprivation impairs host defense. Am J Physiol 1993 (in press).