Sleep as a Prognostic Indicator During Infectious Disease in Rabbits (43590)

LINDA A. TOTH,*[,1] ELIZABETH A. TOLLEY,[†] AND JAMES M. KRUEGER[‡]

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

Abstract. **Infectious disease alters sleep patterns in rabbits, but the recuperative value of enhanced sleep during infectious disease has not been experimentally verified. To evaluate the relationship between specific sleep patterns and the clinical response to infectious disease, we classified sleep patterns in rabbits inoculated with *E. coli*, *S. aureus*, or *C. albicans* on the basis of the duration of the period of enhanced sleep. Patterns characterized by a long period of enhanced sleep were associated with a more favorable prognosis and less severe clinical signs than were patterns characterized by relatively short periods of enhanced sleep followed by prolonged sleep suppression. A contrasting analysis of these data indicated that animals that eventually died demonstrated reduced sleep compared to rabbits that survived the infection. These observations are consistent with the hypothesis that dynamic changes in sleep over the course of an infectious disease aid in recuperation.** [P.S.E.B.M. 1993, Vol 203]

The concept of sleep as an active process mediated by specific brain regions was formulated over 60 years ago by von Economo (1) based on the study of central nervous system lesions produced during viral infections. Until recently, however, the relationship between vigilance and infectious disease received little systematic investigation despite the common observations that many people experience feelings of increased sleepiness during infectious disease and that bed rest has been prescribed by physicians for centuries as an aid in recuperation (2). Current evidence suggests that sleep, like fever, is one of the manifestations of infectious disease known collectively as the acute phase response, and that the physiological systems regulating the immune response, sleep, and body temperature are closely interrelated (3). However, little information is available to address the issue of the clinical relevance of altered sleep during microbial infections. The observation of prolonged periods of increased sleep in rabbits that survived bacterial challenge compared to brief periods of increased sleep in rabbits that eventually died (4) suggested that sleep patterns could provide a prognostic indicator under some conditions and further implied an adaptive function for sleep in combatting infectious disease.

These considerations led us to further investigate the relationship between specific sleep patterns and the clinical response to microbial challenge in rabbits. Two approaches were used in this evaluation. First, the sleep patterns of microbially inoculated rabbits were grouped based on the temporal pattern of sleep enhancement and were evaluated to determine whether sleep pattern was related to clinical outcome. Second, sleep patterns from rabbits that survived the 48-hr postinoculation period were compared to patterns from rabbits that became moribund or died during this period. Data confirm our previous suggestion that a prolonged pattern of increased sleep is associated with a more favorable clinical prognosis after microbial challenge.

Materials and Methods

A total of 93 adult male New Zealand White rabbits (*Pasteurella multocida*- and *coccidia*-free; Myrtle's Rabbitry, Thompson Station, TN), weighing 4–5 kg, were used in these experiments. Data from most of these rabbits have been previously reported in another form as part of other studies (4–6).

Rabbits were surgically implanted with electroencephalographic (EEG) recording electrodes and brain thermistors, as previously described (7), and were al-

[1] To whom requests for reprints should be addressed at: Department of Infectious Diseases, Comparative Medicine Division, St. Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, TN 38105.

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lowed to recover for several weeks before being used experimentally. Rabbits were housed individually on a 12 hr:12 hr light-dark cycle, with lights on at 0600 hr, in a temperature-controlled room (21 ± 2°C). Before testing, rabbits were moved to a sound-attenuated temperature-controlled chamber maintained under the same environmental conditions as the home cage and were permitted an overnight period of adaptation. Baseline sleep patterns were then recorded for 24 to 48 hr before the rabbits received any experimental treatment (Day 1). Rabbits then received intravenous inoculation with *Escherichia coli*, *Staphylococcus aureus*, or *Candida albicans*, as described below, and recording continued for an additional 48 hr. Blood samples and rectal temperatures were taken at 0730 hr throughout the experimental period. During the recording period, rabbits were able to move freely in their cages and had continuous access to food and water.

EEG and brain temperature were continuously recorded via a rotary commutator that permitted unrestricted movement by the animal. Animal movement was monitored via a Grass acceleration transducer (Grass Instruments, Quincey, MA) connected to the commutator cable. Delta (0.5–4.0 Hz) wave components of the EEG were quantified using band-pass filters (Buxco Electronics, Sharon, CT, and Coulbourn Instruments, Lehigh Valley, PA) and an analog to digital converter. The analog signals, the filtered rectified delta component, animal movement, and brain temperature were displayed on a Grass polygraph. In addition, average EEG delta wave amplitudes (DWA) were stored in digital form on computer for each 1-min interval of recording.

Periods of slow wave sleep (SWS) are associated with an increased amplitude of low frequency (delta) EEG waves and the absence of body movement (7). On this basis, the EEG tracing, the filtered and rectified EEG delta wave signal, and the movement recording were visually examined for the first 6-hr period of the baseline recording period to determine a threshold DWA associated with SWS for each animal. The data for each animal were then scored in 1-min intervals for the entire experiment. An animal was considered to be in a state of SWS whenever the average DWA for any interval exceeded the SWS threshold in the absence of movement. At other times, the animal was either awake or in rapid eye movement sleep (REMS). REMS was identified by visual assessment of the polygraph record based on the criteria of a low voltage EEG tracing, a rise in brain temperature, and the sporadic occurrence of phasic body movement (7). Data were summarized for every 2-hr interval. Sleep parameters evaluated were the percentage of time spent in SWS, DWA during SWS, the number and length of individual bouts of SWS, and the number of minutes spent in REMS.

Sleep patterns of individual rabbits were evaluated in terms of a sleep quality score (SQS) that was calculated for each 2-hr period of the experiment. The SQS was calculated as the product of the percent time in SWS and the DWA during SWS (expressed as a percentage of baseline values) divided by 100, and thus reflects both the duration and the intensity of SWS (8). Data are presented as the mean difference between values obtained during baseline and experimental periods for individual rabbits and as the cumulative SQS score over the 72-hr recording period.

For the preparation of microbial inocula, *S. aureus* (American Type Culture Collection [ATCC; Rockville, MD] 29213), *E. coli* (ATCC 25922), and *C. albicans* (ATCC 310) were purchased as lyophilized cultures on Culti-loops (Scott Laboratories, Fiskeville, RI). Prewarmed blood agar or Sabaroud's plates were inoculated and incubated for 24 hr at 37°C. Colonies were then transferred to sterile pyrogen-free saline to achieve a concentration of approximately 2×10^8 colony-forming units/ml, estimated using a Klett-Summerson photoelectric colorimeter. Rabbits were inoculated intravenously in the marginal ear vein with 0.1–0.5 ml of this microbial suspension, which was estimated to contain approximately 10^7-10^8 colony-forming units of *E. coli*, *S. aureus*, or *C. albicans*. The specific dosage administered to each rabbit was subsequently determined by plating serial dilutions of the inoculation suspension on blood agar plates and counting the number of colonies 24–48 hr later. The dosages of infectious agents used in these studies were intended to produce moderately severe clinical disease from which the animals would eventually recover. In no instance were animals purposefully injected with dosages intended to result in death. Each rabbit was inoculated only once and was euthanatized at the end of the experiment with intravenous T61 euthanasia solution (Hoechst-Roussel, Somerville, NJ).

Blood samples (3 ml) were collected from the central ear artery and immediately transferred to vacuum tubes containing EDTA or citrate. Total white blood cell (WBC) counts were measured using a model 2N cell counter (Coulter Electronics, Hialeah, FL). Differential WBC counts were made by counting 100 WBCs from blood smears stained with Wright stain; final WBC counts were corrected for nucleated red blood cells (nRBC), if present. Fibrinogen concentrations were measured using a fibrometer (Becton-Dickinson, Towson, MD). Plasma triglyceride concentrations were measured using a Reflotron clinical chemistry analyzer (Boehringer-Mannheim Diagnostics, Indianapolis, IN). Plasma cortisol concentrations were measured using a RIA kit (Kallestad Labs, Austin, TX). These parameters are commonly used in clinical studies to estimate the severity of the disease state or to monitor the progression of infectious processes (9, 10). The hematological and other pathophysiological changes induced by the

administration of microbial agents in these experiments are qualitatively and quantitatively similar to those observed in naturally occurring disease in rabbits (11) or in rabbits inoculated with natural pathogens by normal routes of infection (12).

Most of the rabbits used in these experiments were euthanatized at 48 hr postinoculation. However, for humane reasons, several animals were euthanatized earlier due to the development of a moribund condition characterized by extreme behavioral depression with unresponsiveness to handling and other stimuli, peripheral vasoconstriction, as evidenced by cold extremities, and, in some cases, dyspnea; the sleep patterns of these animals were unknown at the time of euthanasia. Because their spontaneous death was imminent, animals that were euthanatized before 48 hr postinoculation due to a moribund condition were considered part of the group of animals that died. Postmortem blood cultures were performed using samples obtained by cardiac puncture immediately after death. Blood was incubated for 48 hr at 37°C in brain-heart infusion broth. An aliquot was then transferred to blood agar (*E. coli*- and *S. aureus*-inoculated rabbits) or Sabaroud's agar (*C. albicans*-inoculated rabbits) for an additional 24-hr incubation.

The data collected in these experiments were analyzed to permit an evaluation of the relationship between the sleep alterations that developed during infectious disease and the clinical outcome of the infectious condition. Two approaches were used in this evaluation. First, sleep patterns of individual rabbits inoculated with *E. coli*, *S. aureus*, or *C. albicans* were initially evaluated based on the temporal pattern of SQS scores plotted as a difference from baseline values (Fig. 1). Individual rabbits were assigned to groups, designated minimal sleep changes (MS), enhanced sleep (ES), or suppressed sleep (SS), based on the duration of the SQS increase, as summarized in Table I. This analysis permitted a determination of the predictive value of the sleep pattern in terms of clinical outcome. In this evaluation, SQS scores, other sleep parameters, temperature data, and clinical parameters were analyzed initially using either three-way (sleep variables) or two-way (clinical variables) analysis of variance for repeated measures over days and hours. When no significant differences were detected between groups for the baseline day, data from all groups were combined to give more precise estimates of hourly means. Subsequently, all responses on postinoculation days were compared to those on the baseline day using either three-way analysis of variance with rabbits as blocks (sleep variables) or one-way analysis of variance (clinical variables). Because the between-animal and within-animal variations were pooled for clinical variables, tests of hypothesis should be regarded as conservative. In all cases, *a priori* comparisons among means were made with Fisher's least significant difference test (13). In the second approach, data were analyzed by comparing sleep patterns from rabbits that survived the 48-hr postinoculation period to patterns obtained from rabbits that became moribund or died during this period. In this analysis, cumulative SQS scores were calculated for 22-hr periods on the baseline day and on each day postinoculation. Data were analyzed nonparametrically using Fisher's exact test to compare proportions of animals exhibiting cumulative SQS values outside the 80% or 90% confidence intervals determined on the baseline day of recording. To complement this analysis,

Table I. Sleep Patterns Associated with *E. coli*, *S. aureus*, or *C. albicans* Inoculation in Rabbits: Relationship to Dose, Blood Cultures, and Mortality

Microbe	Sleep pattern	SQS increase (hr duration)	*n*	Dose ($\times 10^7$ CFU)	Positive blood cultures[a]	Mortality[a]
E. coli	Enhanced sleep	≥4	7	3.8 ± 2.8	2/7 (12)[b]	0/7 (0)
	Suppressed sleep	<4	12	12.2 ± 2.1[c]	9/12 (75)	8/12 (67)[c]
S. aureus	Minimal change	Minimal	8	2.3 ± 1.3	1/8 (12)[d]	0/8 (0)
	Enhanced sleep	≥16	26	4.8 ± 0.7	8/17 (47)	5/26 (19)
	Suppressed sleep	<16	11	8.7 ± 1.1[c]	4/9 (44)	7/11 (63)[c]
C. albicans	Minimal change	Minimal	9	3.9 ± 1.1	3/8 (38)[e]	0/9 (0)
	Enhanced sleep	≥14	13	5.3 ± 0.9	8/11 (73)	0/13 (0)
	Suppressed sleep	<14	7	6.8 ± 1.3	4/6 (67)	1/7 (14)

SQS, Sleep quality score or [(% time in SWS) × (% baseline DWA during SWS)]/100; hr duration, duration of increase in hours; CFU, colony-forming units. *P*-values were calculated using Fisher's exact test (blood cultures, mortality) or Student's *t* test (dose), and refer to comparison with other groups inoculated with the same microorganism.

[a] Percentages are in parentheses.

[b] $P = 0.067$.

[c] $P < 0.02$.

[d] $P = 0.094$.

[e] $P = 0.13$.

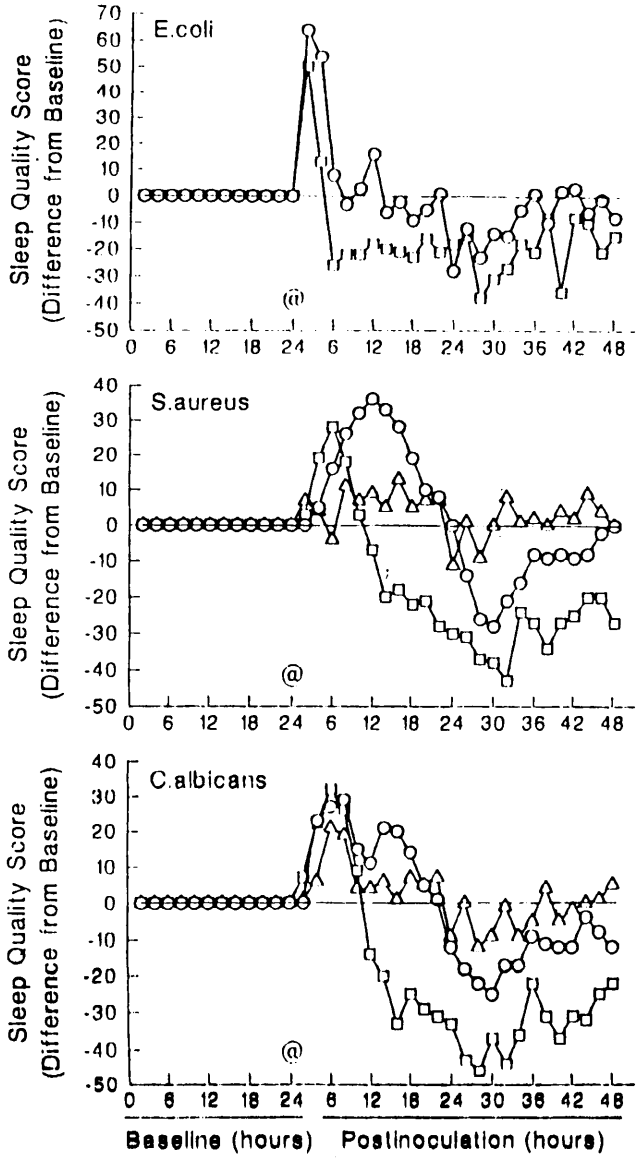

Figure 1. SQS values after microbial inoculation in rabbits. Panels represent SQS from rabbits inoculated with *E. coli* (top), *S. aureus* (middle), and *C. albicans* (lower). SQS is calculated as [(% time in SWS) × (% baseline DWA during SWS)]/100 and is expressed as a difference from baseline (Day 1) values. The solid line represents extrapolation of baseline values from Day 1. For all panels, triangles represent the MS group, circles represent the ES group, and squares represent the SS group. @, The time of microbial inoculation.

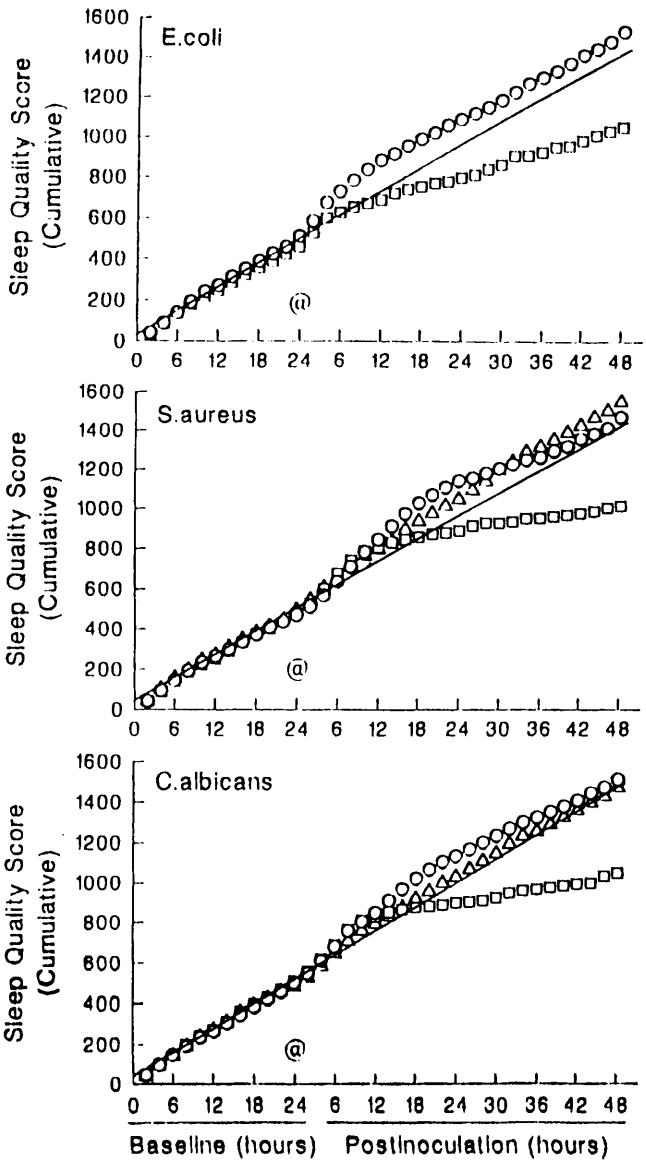

Figure 2. CSQS after microbial inoculation in rabbits. Panels represent CSQS from rabbits inoculated with *E. coli* (top), *S. aureus* (middle), and *C. albicans* (lower). SQS is calculated as [(% time in SWS) × (% baseline DWA during SWS)]/100. SQS values from each 2-hr recording interval were sequentially summed over the course of the experiment to provide CSQS values. The solid line represents extrapolation of baseline values from Day 1. For all panels, triangles represent the MS group, circles represent the ES group, and squares represent the SS group. @, The time of microbial inoculation. Significant differences ($P < 0.05$) were detected during the postinoculation period as follows: *E. coli*-inoculated rabbits, ES > SS from 2 to 48 hr; *S. aureus*-inoculated rabbits, ES > SS from 14 to 48 hr, ES > MS from 14 to 24 hr, ES < MS from 44 to 48 hr, MS > SS from 20 to 48 hr; *C. albicans*-inoculated rabbits, ES > SS from 16 to 48 hr, ES > MS from 14 to 32 hr, MS > SS from 22 to 48 hr.

paired *t* tests and Friedman's rank test were used to compare cumulative SQS obtained during the 6 hr before death to cumulative values obtained during the same postinoculation interval in rabbits that survived comparable doses of the same infectious agent.

Results

Sleep Patterns after Microbial Inoculations: Relationship to Mortality. Inoculation of rabbits with *S. aureus* or *C. albicans* induced one of three patterns of SQS changes, designated MS, ES, and SS patterns based on the duration of the initial increase in SQS; only ES and SS patterns were identified in *E. coli*-inoculated rabbits (Table I). All three groups demonstrated biphasic changes in SQS, although the initial increase in this parameter was greater in duration and/or magnitude in the ES groups and was minimal in the MS groups (Fig. 1).

Cumulative SQS (CSQS) from rabbits before and

after microbial inoculation are illustrated in Figure 2. In rabbits inoculated with *E. coli*, the CSQS of the SS groups increased during the first 2 hr postinoculation, but thereafter accumulated at a rate that was markedly slower than that observed during the baseline period. In contrast, the CSQS of ES rabbits initially increased at a more rapid rate and for a longer time than did the score of SS rabbits. The rate of accumulation then decreased and returned to a basal rate at approximately 24 hr postinoculation. The CSQS values of the SS group were significantly lower than those of the ES group from 2 to 48 hr postinoculation ($P < 0.05$). In rabbits inoculated with *S. aureus*, the initial rate of SQS accumulation increased to a similar degree in all three groups of rabbits for approximately 12 hr after inoculation. Subsequently, the rate was markedly reduced from 12 to 48 hr postinoculation for the SS group, such that CSQS values for SS rabbits were significantly less than those in the ES group from 14 to 48 hr or in the MS group from 20 to 48 hr postinoculation ($P < 0.05$). The ES group demonstrated an increased rate of accumulation for approximately 24 hr postinoculation, and CSQS values for ES rabbits were significantly higher than values for the MS group from 14 to 24 hr ($P < 0.05$). The rate of accumulation in the ES group was then decreased for the next 12 hr and returned to the basal rate for the remaining 12 hr. CSQS values from the ES group were significantly lower than those in the MS group from 44 to 48 hr postinoculation ($P < 0.05$). This difference reflected the late phase of SQS suppression in the ES group; the MS group did not demonstrate a marked phase of SQS suppression after the initial increase (Fig. 1). In *C. albicans*-inoculated rabbits, the initial rate of SQS accumulation increased similarly in the ES and SS groups of rabbits for approximately 10 h after inoculation (Fig. 2). The rate was then markedly reduced from approximately 14 to 48 hr postinoculation for the SS group, such that CSQS values for SS rabbits were significantly less than those in the ES group from 16 to 48 hr or in the MS group from 22 to 48 hr postinoculation ($P < 0.05$). The ES group demonstrated an increased rate of accumulation for at least 24 hr postinoculation, and CSQS values for ES rabbits were significantly higher than values in the MS group from 14 to 32 hr postinoculation ($P < 0.05$). The rate of accumulation in the ES group then decreased and returned to the basal rate during the second day postinoculation. Relative to changes observed in ES and SS groups, the MS group demonstrated a minimally biphasic patterns (Fig. 1).

The alterations in SQS that were observed for each group reflected parallel changes in SWS and DWA during SWS. The sleep alterations induced by *E. coli* inoculation were characterized by a significant increase in the amount of time in SWS from 0 to 4 hr postinoculation for both SS and ES rabbits (Fig. 3A). The

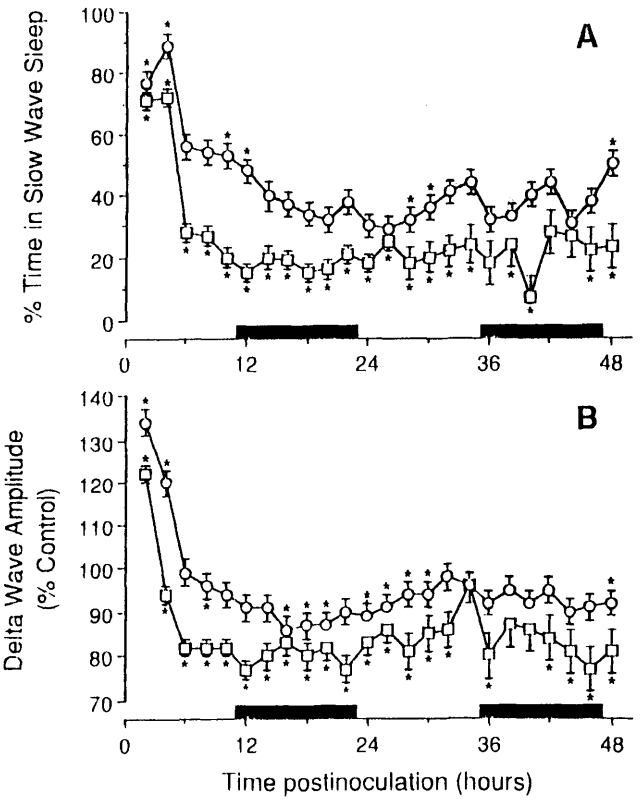

Figure 3. Alterations in SWS and DWA during SWS in rabbits inoculated with *E. coli*. Upper panel, Percentage of time in SWS; lower panel, DWA during SWS (expressed as a percentage of baseline values). For both panels, circles represent the ES group, and squares represent the SS group. Baseline values obtained for individual groups were not significantly different and were combined for statistical analysis. The shaded area represents the baseline values; these values are repeated during the second 24-hr interval to facilitate visual comparisons. The horizontal bars along the *x* axis indicate the lights-off period. During the baseline period, circadian variations in the amount of time spent in SWS or DWA during SWS are evident; for both parameters, values are highest during the daylight hours and lower during the dark period, with the lowest values occurring at dusk and dawn. *$P < 0.03$ relative to comparable baseline values.

period of increased sleep was characterized by increased DWA during SWS (Fig. 3B), increased SWS bout length, and a reduced number of SWS bouts (data not shown). The magnitude of these changes was greater in all cases for the ES group than for the SS group. SWS time was reduced below corresponding baseline values from 8 to 48 hr postinoculation for the SS rabbits. For the ES group, a significant increase in SWS time persisted for up to 12 hr; subsequent decreases in this parameter over the next 18 hr were sporadic and modest in magnitude relative to the decreases observed in the SS group. The phase of decreased SWS was characterized by reductions in DWA during SWS and in the number of SWS bouts. These changes were greater in magnitude and duration for the SS group. REMS was reduced throughout the postinoculation period to a similar degree in both ES and SS rabbits (data not shown).

The specific sleep alterations induced by *S. aureus*

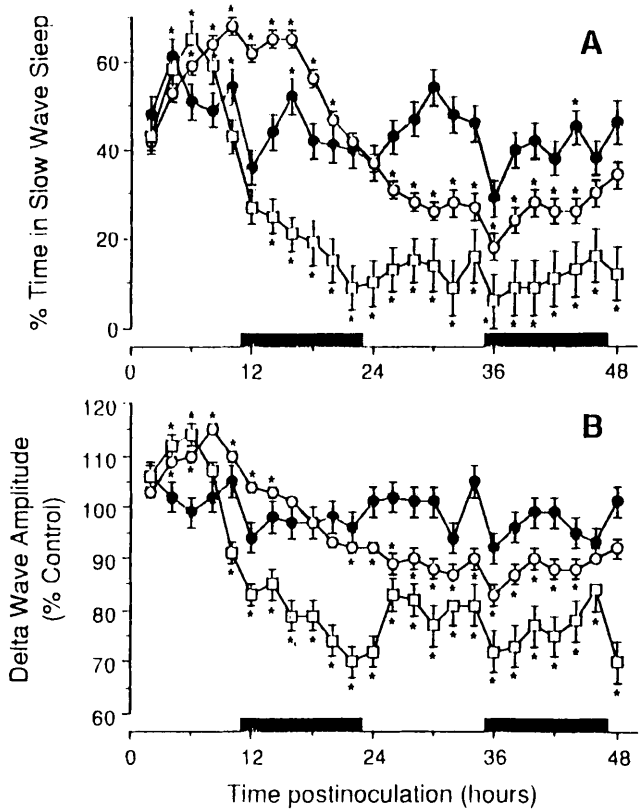

Figure 4. Alterations in SWS and DWA during SWS in rabbits inoculated with *S. aureus*. Upper panel, Percentage of time in SWS; lower panel, DWA during SWS (expressed as a percentage of baseline values). For both panels, filled circles represent the MS group, open circles represent the ES group, and squares represent the SS group. Baseline values obtained for individual groups were not significantly different and were combined for statistical analysis. The shaded area represents the baseline values; these values are repeated during the second 24-hr interval to facilitate visual comparisons. The horizontal bars along the *x* axis indicate the lights-off period. During the baseline period, circadian variations in the amount of time spent in SWS or DWA during SWS are evident; for both parameters, values are highest during the daylight hours and lower during the dark period, with the lowest values occurring at dusk and dawn. $^*P < 0.03$ relative to comparable baseline values.

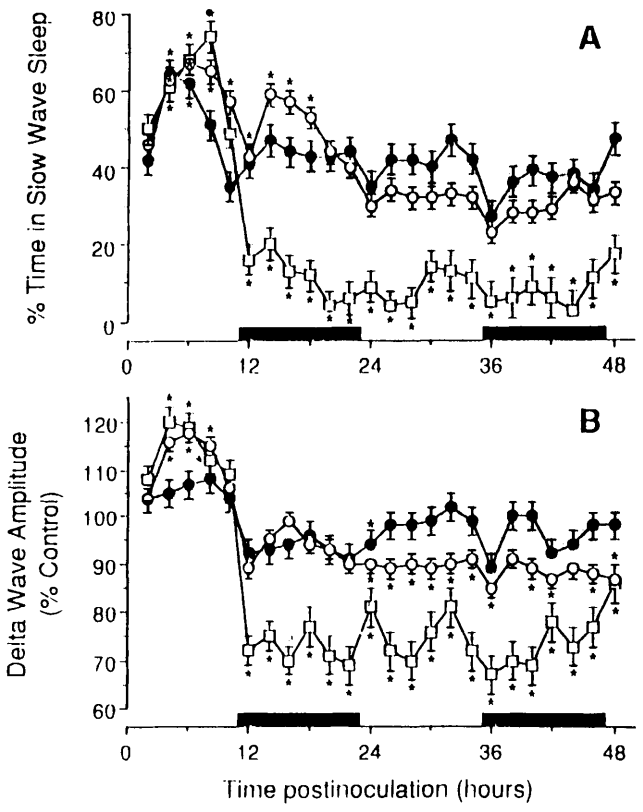

Figure 5. Alterations in SWS and DWA during SWS in rabbits inoculated with *C. albicans*. Upper panel, Percentage of time in SWS; lower panel, DWA during SWS (expressed as a percentage of baseline values). For both panels, filled circles represent the MS group, open circles represent the ES group, and squares represent SS group. Baseline values obtained for individual groups were not significantly different and were combined for statistical analysis. The shaded area represents the baseline values; these values are repeated during the second 24-hr interval to facilitate visual comparisons. The horizontal lines above the *x* axis indicate the lights-off period. During the baseline period, circadian variations in the amount of time spent in SWS or DWA during SWS are evident; for both parameters, values are highest during the daylight hours and lower during the dark period, with the lowest values occurring at dusk and dawn. $^*P < 0.03$ relative to comparable baseline values.

inoculation also paralleled the changes in SQS scores (Fig. 4). The MS pattern was characterized by sporadic increases in SWS from 4 to 16 hr postinoculation (Fig. 4). Other SWS indices were not significantly altered, although REMS showed occasional significant reductions throughout the 48-hr postinoculation period (data not shown). In contrast to the MS pattern, the ES pattern was characterized by increased time in SWS from 6 to 22 hr postinoculation, increased DWA during SWS from 4 to 14 hr, and increased SWS bout length during SWS from 8 to 16 hr (data not shown). Subsequent reductions in these parameters occurred from 26 to 44 hr postinoculation for SWS time, 22 to 48 hr for DWA during SWS, and 28 to 30 hr for SWS bout length. REMS was reduced from 4 to 46 hr postinoculation in the ES group (data not shown). In the SS group, time in SWS was significantly increased from 6 to 8 hr postinoculation and was reduced from 14 to 48 hr. Changes in DWA during SWS paralleled this effect, with increased values from 4 to 6 hr postinoculation and reduced values from 10 to 48 hr. SWS bout length was moderately reduced, although this effect was not statistically significant (data not shown). However, the number of individual bouts of SWS was significantly reduced from 18 to 48 hr postinoculation (data not shown). REMS was reduced throughout the 48-hr postinoculation period (data not shown).

The specific sleep alterations induced by *C. albicans* inoculation also paralleled the changes in SQS scores (Fig. 5). The MS pattern was characterized by increased SWS time (Fig. 5) and bout length (data not shown) from 6 to 8 hr postinoculation. DWA and the number of bouts of SWS were not significantly altered, but REMS was moderately reduced throughout the 48-hr postinoculation period (data not shown). In contrast

to the MS pattern, the ES pattern was characterized by increased time in SWS from 4 to 18 hr postinoculation, increased DWA during SWS, and increased SWS bout length (data not shown) from 4 to 8 hr. Subsequent reductions in these parameters occurred from 24 to 42 hr postinoculation for SWS time, from 24 to 48 hr for DWA during SWS, and from 22 to 48 hr for SWS bout length. The number of bouts of SWS was reduced sporadically, but REMS was virtually absent from 4 to 48 hr postinoculation (data not shown). In the SS group, time in SWS was significantly increased from 4 to 8 hr postinoculation and was reduced from 12 to 48 hr. Changes in DWA during SWS paralleled this effect, with increased values from 4 to 8 hr postinoculation and reduced values from 12 to 48 hr. SWS bout length was reduced from 12 to 46 hr postinoculation, and the number of individual bouts of SWS was reduced from 16 to 48 hr postinoculation (data not shown). REMS was reduced throughout the 48-hr postinoculation period (data not shown).

Clinical Sequelae Associated with Specific Sleep Patterns after Microbial Inoculation. The clinical sequelas of *E. coli* inoculation are shown in Table II. A significant increase in body temperature was not present in either group of rabbits 24 hr postinoculation, although SS rabbits were febrile at 48 hr postinoculation. Inoculation with *E. coli* did not significantly alter total WBC counts, but did induce neutrophilia and lymphopenia; these effects were similar in both ES and SS rabbits, although the lymphopenia persisted longer in the SS rabbits. Circulating nRBC and plasma concentrations of triglycerides were significantly increased in SS rabbits, but not in ES rabbits. Plasma cortisol concentrations were increased in both groups of rabbits inoculated with *E. coli*, although this increase persisted longer in the SS group. Plasma fibrinogen was not significantly elevated in either group of rabbits.

S. aureus induced fevers of 0.8–1.5°C at 24 hr postinoculation in all groups of rabbits, although the fever persisted until 48 hr only in the ES group (Table III). Significant leukopenia developed only in the SS group. Significant neutrophilia was detected at 24 and 48 hr in the ES group and at 48 hr postinoculation in the SS group, but did not develop in the MS group. In contrast, lymphopenia was present at both 24 and 48 hr postinoculation in all three groups of rabbits, although this effect was of lesser magnitude in the MS group. ES and SS groups both demonstrated significant increases in numbers of circulating nRBCs and in plasma concentrations of fibrinogen, cortisol, and triglycerides; the latter two parameters were increased by a greater degree in the SS group.

Rabbits inoculated with *C. albicans* developed fevers of 1.2–1.4°C within 24 hr postinoculation, and fevers persisted until 48 hr postinoculation (Table IV). Significant leukopenia was present in all three groups at 24 hr postinoculation, but persisted for 48 hr only in the ES group. Significant neutrophilia was detected at 24 and 48 hr in the ES group and at 24 hr in the MS group, but did not develop in the SS group. In contrast, lymphopenia was present at both 24 and 48 hr postinoculation in all three groups of rabbits. Cortisol concentrations were increased in all three groups of rabbits, although this effect was only significant at 24 hr postinoculation in the MS group. The MS group did not demonstrate significant alterations in the other clinical parameters. The SS group, but not the ES group, demonstrated significant increases in numbers of circulating nRBCs and in plasma concentrations of triglycerides. Fibrinogen concentrations were increased at 24 and 48 hr postinoculation for both ES and SS groups.

Analyses of specific dosage of microorganism administered and the clinical outcome of the infectious condition are summarized in Table I. *E. coli*-inoculated rabbits that demonstrated SS patterns received significantly higher dosages than rabbits with ES patterns, and a significantly larger proportion of the SS rabbits died as a result of the infection. The SS sleep pattern

Table II. Clinical Effects of *E. coli* Inoculation in Rabbits

Parameter	Baseline	ES		SS	
		24 hr	48 hr	24 hr	48 hr
Temperature (°C)	38.8 ± 0.2	39.1 ± 0.3	38.8 ± 0.3	39.0 ± 0.3	40.0 ± 0.4[a]
WBC (% of control)	100 ± 13	136 ± 22	144 ± 22	120 ± 20	154 ± 29
Neutrophils (% of control)	100 ± 30	340 ± 50[a]	309 ± 50[a]	264 ± 47[a]	325 ± 66[a]
Lymphocytes (% of control)	100 ± 6	53 ± 10[a]	80 ± 10	39 ± 9[a]	64 ± 13[a]
nRBC (no./100 WBC)	0.6 ± 5.1	14.3 ± 8.5	4.4 ± 8.5	40.2 ± 7.9[a]	5.5 ± 11.2
Triglycerides (mg/dl)	196 ± 186	520 ± 492	209 ± 492	1686 ± 220[a]	1588 ± 348[a]
Fibrinogen (mg/dl)	284 ± 74	650 ± 196	ND	448 ± 98	513 ± 113
Cortisol (μg/dl)	3.5 ± 1.5	15.9 ± 2.4[a]	6.4 ± 2.4	13.0 ± 2.4[a]	12.9 ± 3.2[a]

ND, Not determined. The numbers of WBC, neutrophils, and lymphocytes measured during the baseline period were 8306 ± 874, 2583 ± 389, and 5101 ± 470/μl of blood, respectively.

[a] $P < 0.03$ relative to baseline values. Baseline values obtained for individual groups were not significantly different and were combined for statistical analysis.

Table III. Clinical Effects of *S. aureus* Inoculation in Rabbits

Parameter	Baseline	MS		ES		SS	
		24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Temperature (°C)	38.9 ± 0.1	39.7 ± 0.2[a]	39.3 ± 0.2	40.6 ± 0.1[a]	40.2 ± 0.1[a]	40.5 ± 0.1[a]	39.3 ± 0.3
WBC (% of control)	100 ± 4	106 ± 10	107 ± 10	93 ± 11	100 ± 14	60 ± 11[a]	93 ± 14
Neutrophils (% of control)	100 ± 13	158 ± 32	151 ± 32	256 ± 16[a]	224 ± 20[a]	157 ± 34	237 ± 42[a]
Lymphocytes (% of control)	100 ± 3	62 ± 7[a]	79 ± 7[a]	26 ± 4[a]	45 ± 5[a]	16 ± 8[a]	31 ± 10[a]
nRBC (no./100 WBC)	0.6 ± 1.1	0.1 ± 2.7	0.5 ± 2.7	5.2 ± 1.5[a]	8.9 ± 1.8[a]	8.2 ± 3.1[a]	14.5 ± 3.8[a]
Triglycerides (mg/dl)	140 ± 67	148 ± 116	146 ± 116	162 ± 97	450 ± 109[a]	626 ± 137[a]	961 ± 154[a]
Fibrinogen (mg/dl)	260 ± 35	300 ± 57	359 ± 61	556 ± 47[a]	874 ± 57[a]	594 ± 75[a]	885 ± 75[a]
Cortisol (μg/dl)	4.6 ± 1.2	8.8 ± 2.8	6.7 ± 2.8	14.9 ± 1.6[a]	11.6 ± 2.1[a]	24.7 ± 3.3[a]	35.4 ± 4.0[a]

The numbers of WBC, neutrophils, and lymphocytes measured during the baseline period were 8488 ± 547, 2495 ± 195, and 5442 ± 308/μl of blood, respectively.
[a] $P < 0.03$ relative to baseline values. Baseline values obtained for individual groups were not significantly different and were combined for statistical analysis.

Table IV. Clinical Effects of *C. albicans* Inoculation in Rabbits

Parameter	Baseline	MS		ES		SS	
		24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Temperature (°C)	38.9 ± 0.1	40.1 ± 0.2[a]	39.9 ± 0.2[a]	40.1 ± 0.1[a]	39.6 ± 0.2[a]	40.3 ± 0.2[a]	39.8 ± 0.2[a]
WBC (% of control)	100 ± 4	82 ± 8[a]	88 ± 8	76 ± 6[a]	78 ± 6[a]	63 ± 8[a]	86 ± 9
Neutrophils (% of control)	100 ± 21	254 ± 37[a]	204 ± 37	265 ± 32[a]	228 ± 32[a]	153 ± 42	173 ± 45
Lymphocytes (% of control)	100 ± 3	40 ± 6[a]	71 ± 6[a]	30 ± 5[a]	41 ± 5[a]	19 ± 7[a]	36 ± 7[a]
nRBC (no./100 WBC)	0.4 ± 0.6	1.9 ± 1.0	2.0 ± 1.0	2.3 ± 0.9	2.5 ± 0.9	4.3 ± 1.2[a]	12.8 ± 1.3[a]
Triglycerides (mg/dl)	134 ± 70	132 ± 118	218 ± 118	212 ± 145	295 ± 145	654 ± 118[a]	1327 ± 130[a]
Fibrinogen (mg/dl)	260 ± 35	300 ± 57	359 ± 61	556 ± 47[a]	874 ± 57[a]	594 ± 75[a]	885 ± 75[a]
Cortisol (μg/dl)	4.4 ± 1.3	11.3 ± 2.2[a]	9.4 ± 2.2	14.9 ± 2.0[a]	11.8 ± 2.1[a]	16.7 ± 2.8[a]	18.0 ± 2.6[a]

The numbers of WBC, neutrophils, and lymphocytes measured during the baseline period were 8671 ± 691, 2138 ± 153, and 6081 ± 323/μl of blood, respectively.
[a] $P < 0.03$ relative to baseline values. Baseline values obtained for individual groups were not significantly different and were combined for statistical analysis.

was also associated with higher dosages and mortality in *S. aureus*-inoculated rabbits. A similar relationship of dosage to sleep pattern was observed for rabbits inoculated with *C. albicans*, although mortality was minimal in this group. The numbers of rabbits in each group that demonstrated positive blood cultures at the end of the experiment did not differ significantly (Table I), although the clear tendency to greater proportions of positive animals in ES and SS groups relative to MS groups suggests that the failure to attain statistical significance reflects a lack of power (Type II error). The percentage of surviving animals as a function of the time after inoculation is illustrated in Figure 6. Deaths began to occur shortly after or coincident with the abrupt decline in SWS and DWA. Although death was not prevalent after *C. albicans* inoculation even in rabbits demonstrating the SS pattern, subjective observation of the clinical condition of surviving SS rabbits at the end of the 48-hr postinoculation period suggested that many of these animals would have spontaneously died within the next 24 hr had they not been euthanatized (see also Table VII).

Mortality after Microbial Inoculation: Relationship to Sleep Tendency. In the second approach to analysis of these data, SQS of animals that became moribund or died were compared to those of rabbits that survived the infection. After *E. coli* inoculation, the mean CSQS of rabbits that eventually died were significantly lower than those of rabbits that survived when compared throughout the initial 22 hr after inoculation ($P < 0.03$, paired t test). From 6 to 14 hr postinoculation, the CSQS of *E. coli*-inoculated rabbits that eventually survived the infection were significantly greater than the upper limit of the 80% confidence interval measured during the baseline period, indicating that they had exhibited enhanced sleep during this period (P values ranging between 0.072 and 0.0005, Fisher's exact test). In contrast, the CSQS scores of rabbits that eventually died were markedly below the lower limit of the same confidence interval from 10 to

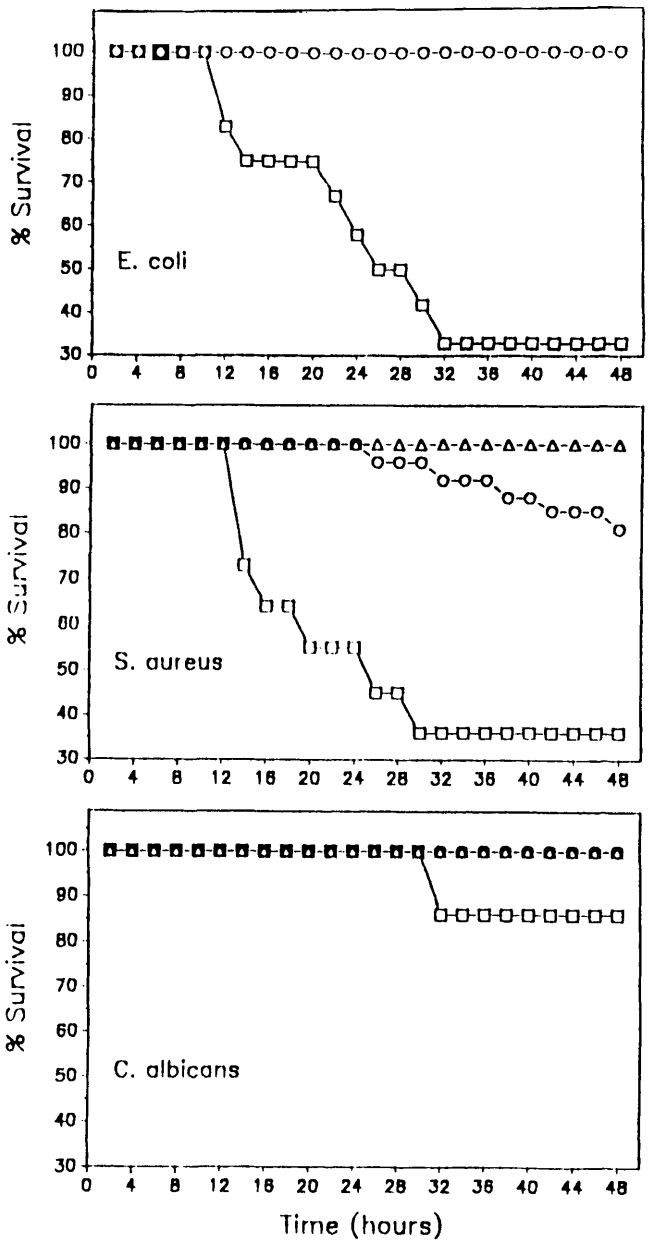

Figure 6. Survivability in rabbits inoculated with *E. coli*, *S. aureus*, or *C. albicans*. Data points indicate the percentage of surviving animals as a function of the postinoculation interval. For all microbes, triangles represent the MS group, circles represent the ES group, and squares represent the SS group. None of the animals in the MS groups died.

22 hr postinoculation, indicating sleep suppression (P values ranging between 0.075 and 0.0002, Fisher's exact test). Finally, mean CSQS values during the 6 hr before death were 50% lower than values obtained during the comparable time period from dosage-matched animals that survived, although these differences did not obtain statistical significance (42 ± 26 vs 83 ± 26; $n = 5$; $P = 0.208$, Friedman's rank test). Relative to rabbits with CSQS within or above the 90% confidence interval for the baseline period, rabbits with CSQS below this confidence interval at 22 hr postinoculation demonstrated a greater increase in circulating nRBC, a less marked neutrophilia, and higher plasma cortisol concentrations in blood samples collected 24 hr after inoculation (Table V). These differences are consistent with a more severe clinical condition in the animals demonstrating sleep suppression, in that a comparison of values obtained from rabbits that died relative to those from rabbits that lived revealed similar changes in these parameters (Table VI). At 48 hr postinoculation, several clinical parameters differed between animals with CSQS below vs within the baseline 90% confidence interval (Table VII). Animals demonstrating sleep suppression were febrile, were not leukophilic, had higher numbers of circulating nRBC, were more lymphopenic, and had higher levels of cortisol, thereby indicating a more severe clinical condition than that observed in animals demonstrating normal or enhanced SQS.

CSQS from *S. aureus*-inoculated rabbits that eventually died were significantly above the upper limit of the 80% confidence interval obtained during the baseline day at 6 hr after inoculation compared to values obtained from rabbits that survived the infection, indicating an early period of sleep enhancement in the group that died ($P = 0.028$, Fisher's exact test). From 8 to 14 hr postinoculation, the CSQS was relatively lower in the group that died, falling below the lower limit of the 90% confidence interval obtained during the baseline period, thereby indicating a period of relative sleep suppression ($P < 0.07$, Fisher's exact test). Sleep suppression relative to the 90% confidence interval was also observed during hours 2–6 on the second day postinoculation in animals that eventually died ($P < 0.06$, Fisher's exact test). These data indicate that the sleep patterns in animals that eventually died demonstrated a biphasic pattern that was temporally accelerated relative to that in animals that survived. Thus, animals that died demonstrated an earlier and less prolonged increase in CSQS relative to baseline than did animals that survived and quickly progressed into a prolonged phase of decreased SQS. The mean CSQS values during the 6 hr before death in *S. aureus*-inoculated rabbits were 80% lower than values obtained during the comparable time period from dosage-matched animals that survived (24 ± 14 vs 123 ± 14) and differed significantly when compared using either Friedman's rank test ($P = 0.0011$) or paired t test ($P = 0.0005$). Relative to rabbits with CSQS within or above the 90% confidence interval for the baseline period, rabbits with CSQS below this confidence interval demonstrated marked hypertriglyceridemia and leukopenia in blood samples collected 24 hr after inoculation (Table V). These differences are consistent with a more severe clinical condition in the animals demonstrating sleep suppression, in that a comparison of values obtained from rabbits that died relative to those in rabbits

Table V. Clinical Parameters Measured 24 hr after Inoculation with *E. coli*, *S. aureus*, or *C. albicans* as a Function of CSQS Relative to the Baseline 90% Confidence Interval

Parameter	*E. coli*		*S. aureus*		*C. albicans*	
	Within	Below	Within	Below	Within	Below
Temperature (°C)	39.0 ± 0.4	39.0 ± 0.3	40.5 ± 0.1[a]	40.0 ± 0.4[a]	40.2 ± 0.1[a]	40.1 ± 0.2[a]
WBC (% of control)	144 ± 23	108 ± 20	95 ± 5	51 ± 16[a,b]	78 ± 5[a]	64 ± 9[a]
Neutrophils (% of control)	362 ± 57[a]	245 ± 49[a,b]	233 ± 15[a]	148 ± 52[a]	243 ± 24[a]	198 ± 46[a]
Lymphocytes (% of control)	54 ± 11[a]	44 ± 10[a]	32 ± 4[a]	15 ± 13[a]	33 ± 4[a]	20 ± 8[a]
nRBC (no./100 WBC)	10.8 ± 9.4[a]	44.5 ± 8.1[a,b]	4.5 ± 1.3[a]	8.3 ± 4.6[a]	2.4 ± 0.8[a]	3.8 ± 1.4[a]
Triglycerides (mg/dl)	—	1492 ± 255[a]	160 ± 55	916 ± 136[a,b]	174 ± 99	638 ± 127[a,b]
Fibrinogen (mg/dl)	—	488 ± 87	484 ± 52[a]	542 ± 124[a]	632 ± 58[a]	546 ± 67[a]
Cortisol (μg/dl)	3.4 ± 0.8	11.7 ± 3.4[a,b]	15.1 ± 1.6[a]	17.8 ± 5.4[a]	13.2 ± 1.5[a]	17.0 ± 2.9[a]

Baseline values were not significantly different from those shown in Tables II–IV for animals inoculated with the same organism. The numbers of WBC, neutrophils, and lymphocytes measured during the baseline period are indicated in Tables II–IV. "Within" and "below" refer to animals with CSQS values within or below the baseline 90% confidence interval.
[a] $P < 0.05$ relative to pooled baseline values for animals that survived until 24 hr postinoculation.
[b] $P < 0.05$ relative to animals with CSQS within the baseline 90% confidence interval.

Table VI. Clinical Parameters after Inoculation with *E. coli* or *S. aureus* as a Function of Mortality

Parameter	*E. coli*		*S. aureus*	
	Lived	Died	Lived	Died
Temperature (°C)	38.8 ± 0.2	38.3 ± 0.4	40.4 ± 0.1[a]	40.7 ± 0.2[a]
WBC (% of control)	158 ± 14[a]	44 ± 24[a,b]	93 ± 5	71 ± 11
Neutrophils (% of control)	377 ± 31[a]	86 ± 51[b]	237 ± 14[a]	162 ± 30[a,b]
Lymphocytes (% of control)	52 ± 6[a]	26 ± 11[a]	32 ± 3[a]	24 ± 7[a]
nRBC (no./100 WBC)	11.8 ± 5.7[a]	73.0 ± 9.5[a,b]	3.3 ± 0.8[a]	11.1 ± 1.7[a]
Triglycerides (mg/dl)	1028 ± 240[a]	1955 ± 240[a,b]	225 ± 45	641 ± 142[a,b]
Fibrinogen (mg/dl)	517 ± 66[a]	373 ± 132	463 ± 35[a]	624 ± 106[a]
Cortisol (μg/dl)	14.3 ± 2.0[a]	15.0 ± 3.8[a]	15.7 ± 1.5[a]	12.4 ± 3.2[a]

Baseline values were not significantly different from those shown in Tables II–III for animals inoculated with the same organism. The numbers of WBC, neutrophils, and lymphocytes measured during the baseline period are indicated in Tables II–III.
[a] $P < 0.05$ relative to pooled baseline values.
[b] $P < 0.05$ relative to animals that survived inoculation with the same organism.

Table VII. Clinical Parameters Measured 48 hr after Inoculation with *E. coli*, *S. aureus*, or *C. albicans* as a Function of CSQS Relative to the Baseline 90% Confidence Interval

Parameter	*E. coli*		*S. aureus*		*C. albicans*	
	Within	Below	Within	Below	Within	Below
Temperature (°C)	38.9 ± 0.4	40.1 ± 0.3[a]	39.7 ± 0.2[a]	40.0 ± 0.2[a]	39.7 ± 0.1[a]	39.8 ± 0.2[a]
WBC (% of control)	155 ± 8[a]	106 ± 12	103 ± 4	94 ± 5	86 ± 4[a]	85 ± 5[a]
Neutrophils (% of control)	305 ± 34[a]	320 ± 54[a]	215 ± 20[a]	202 ± 23[a]	215 ± 22[a]	209 ± 26[a]
Lymphocytes (% of control)	89 ± 8	42 ± 12[a,b]	56 ± 6[a]	46 ± 7[a]	61 ± 4[a]	39 ± 5[a,b]
nRBC (no./100 WBC)	3.5 ± 1.6	9.3 ± 2.5[a]	2.4 ± 2.2	14.5 ± 2.6[a,b]	2.2 ± 0.9[a]	6.1 ± 1.0[a]
Triglycerides (mg/dl)	1128 ± 375	—	234 ± 117	747 ± 131[a,b]	255 ± 122	744 ± 122[a,b]
Fibrinogen (mg/dl)	150 ± 86	695 ± 132	555 ± 73[a]	877 ± 73[a,b]	742 ± 55[a]	945 ± 51[a,b]
Cortisol (μg/dl)	6.3 ± 1.7	13.2 ± 2.6[a,b]	8.2 ± 2.1	21.7 ± 2.4[a,b]	8.4 ± 1.4[a]	17.0 ± 1.6[a,b]

Baseline values were not significantly different from those shown in Tables II–IV for animals inoculated with the same organism. The numbers of WBC, neutrophils, and lymphocytes measured during the baseline period are indicated in Tables II–IV. "Within" and "below" refer to animals with CSQS values within or below the baseline 90% confidence interval.
[a] $P < 0.05$ relative to pooled baseline values for animals that survived until 48 hr postinoculation.
[b] $P < 0.05$ relative to animals that survived inoculation with the same organism.

that lived revealed similar changes in these parameters (Table VI). At 48 hr postinoculation, several clinical parameters differed between animals with CSQS below versus within the baseline 90% confidence interval (Table VII). Animals demonstrating sleep suppression had higher numbers of circulating nRBC and higher levels of triglycerides, fibrinogen, and cortisol, thereby indicating a more severe clinical condition that than observed in animals demonstrating normal or enhanced SQS.

Because only one *C. albicans*-inoculated rabbit died during the 48-hr postinoculation period, comparisons similar to those performed above could not be used to evaluate animals inoculated with this pathogen. However, a comparison of clinical parameters from rabbits with CSQS below versus within or above the 90% confidence intervals for CSQS during the control period indicated that animals demonstrating sleep suppression were probably more severely ill than animals demonstrating normal or enhanced sleep (Tables V and VII).

Discussion

The data presented here demonstrate that the clinical outcome of the infectious condition is related to the specific sleep/EEG changes that develop during infection. Animals that respond to microbial challenge with a robust enhancement of the amount of SWS and the amplitudes of EEG slow waves during SWS have a greater probability of surviving than animals that develop a prolonged suppression of sleep. Analogously, animals that eventually died demonstrated suppressed sleep relative to that observed in rabbits that survived the infection. A marked reduction of EEG amplitudes has previously been observed in animals that died as a result of rabies inoculation (14) or chronic sleep deprivation (15, 16). The amplitudes of EEG slow waves during SWS are hypothesized to reflect the depth or intensity of sleep, largely based on observations that these amplitudes increase during the deep sleep that follows sleep deprivation (17–19). The enhanced EEG amplitudes that occur during the initial responses to infection thus suggest that infected animals sleep with a greater intensity than normal.

The sleep pattern was related to the severity of the infectious condition in terms not only of mortality but also of the severity of various clinicopathological indices. Rabbits demonstrating prolonged enhancement of sleep developed fewer or less pronounced changes in the clinical parameters used to gauge the severity of infection, e.g., lymphopenia, triglyceridemia, and cortisolemia were less in the ES groups than in the SS groups. Increases in the numbers of nRBC in the circulation, which is likely to reflect septicemia-induced damage to the bone marrow endothelium (20), was consistently higher in the SS groups. In addition, the degree of neutrophilia that developed in the SS group was in some cases reduced in magnitude or delayed in onset relative to that in the ES group, suggesting an inadequate leukocytic response in the face of an overwhelming microbial challenge. Clinical indices measured in rabbits demonstrating CSQS below the 90% confidence intervals for the baseline period were similarly more severely altered than those obtained from rabbits demonstrating enhanced or normal CSQS.

The data presented indicate that the sleep pattern can reflect the infectious dosage to which the animal was exposed. The experimental paradigm used in these studies is analogous to typical clinical cases of spontaneous infectious processes in which the precise infectious dose is unknown at the time of exposure. The analyses performed after the experiments indicate that animals demonstrating SS patterns had generally received somewhat higher doses of microbial agents than had animals with ES patterns. Thus, our data indicate that a pattern of sleep suppression was suggestive of exposure to a higher infective dose. The use of a range of dosages in this study does not confound the basic observation that sleep pattern is related to clinical outcome, in that even among dosage-matched animals, those animals that died generally exhibited lower CSQS than the animals that survived. Thus, dosage alone is not the only factor involved in predicting outcome. Individual variation in the animal's physiological and immunological responses to a given infectious challenge can result in different outcomes even in animals that received identical dosages; in these cases, the sleep pattern reflects the clinical deterioration. Induction of severe clinical illness, due to either a higher infectious dose or an inadequate defense response by the infected animal, is associated with marked suppression of normal sleep.

Although clinicopathological indicators and mortality are in general directly correlated with dosage, the amount and intensity of SWS decreased in severely affected animals. A similar relationship seems to develop in AIDS victims, in that HIV-seropositive but otherwise healthy individuals exhibit an excessive amount of Stage 4 sleep (the human equivalent of SWS in animals) during the latter half of the night (21), yet sleep deteriorates and becomes disrupted as the disease progresses (22). However, the role of sleep *per se* in mediating the recovery from infectious disease is unknown. Our observations suggest either that impaired sleep reflects the deteriorating clinical condition of the animal, or, alternatively, that enhanced sleep is beneficial for the animal in terms of survivability. According to the former interpretation, sleep could simply provide an index of general well-being during conditions of microbial disease, such that animals that are less severely affected by the infection are able to sleep better. However, an important observation relevant to this

possibility is that despite the varying degrees of severity of the infectious conditions induced in animals in this study, the initial response to the infection was uniformly to sleep more than normal. This observation suggests that dynamic changes in sleep could actively contribute to the recovery process. This hypothesis is indirectly supported by several observations suggesting that immune competence is adversely affected by sleep deprivation. For example, sleep-deprived mice immunized against influenza virus failed to clear the virus from the lungs upon subsequent challenge, although similarly immunized animals that were not sleep deprived completely cleared the virus (23). A related study demonstrated that sleep-deprived rats showed a suppressed secondary antibody response when repeatedly inoculated with sheep red blood cells (24). Other reports have also indicated that sleep deprivation alters certain facets of the immune response (25–29), although negative results have been reported (30). The hypothesis that sleep is important to health is also supported by observations that in rats, the eventual sequel to chronic sleep deprivation is death (15, 16).

The mechanisms by which sleep is altered during infectious disease are likely to involve microbes and their cellular components as well as the immune response that is stimulated in the infected animal. During the process of bacterial phagocytosis and digestion, macrophages release biologically active muramyl peptides from bacterial cell wall peptidoglycan. Dead bacteria (4, 5), bacterial peptidoglycan (31), and supernatants from macrophages incubated with bacteria or bacterial peptidoglycan (32) are all able to elicit sleep responses similar to those described here. Several purified or synthetic muramyl peptides elicit similar responses (7, 33, 34). Other bacterial cell wall products have been implicated in the sleep responses to infection. For example, endotoxin and its lipid A moiety are cell wall components of gram-negative bacteria that also elicit sleep responses (35); interestingly, the sleep responses induced by the intravenous injection of endotoxin have a rapid onset, similar to responses elicited by viable gram-negative bacteria (5, 12).

Bacterial products, such as muramyl peptides and endotoxin, probably induce their effects on sleep and fever via their ability to enhance cytokine production. Microbial challenge stimulates a host immune response that is characterized by increased production of interleukin-1 (IL-1) and other cytokines. Several of these cytokines, including IL-1 and tumor necrosis factor, enhance sleep when administered intravenously or intracerebroventricularly (36, 37). IL-1 and tumor necrosis factor have been associated with several of the pathophysiological sequelae of infectious disease, and levels of these cytokines have been demonstrated to increase in the circulation during septic disease and other conditions (38–42). The production of these somnogenic cytokines could reflect the adequacy of the host defense response in the infected animal. For example, those animals generating a robust immune response could demonstrate augmentation not only of sleep, but also of other facets of host defense, resulting in a favorable outcome. On the other hand, those animals that generate a weak immune response to the microbial challenge show reduced sleep and are quickly overwhelmed by the infection. However, recent data have indicated that patients who died from septicemia had higher plasma levels of IL-1 and tumor necrosis factor than similar patients who survived (39, 43). Similarly, IL-1 concentrations were higher in the cerebrospinal fluid of infants that died of bacterial meningitis than in infants that survived and were temporally correlated to the detection of endotoxin, bacterial antigens, and positive cerbrospinal fluid cultures (44). High systemic doses of IL-1 induce a syndrome similar to endotoxic shock (45), and the administration of an IL-1 receptor antagonist protects animals from death in models of septic shock (46, 47). Interestingly, studies in rats indicate that low doses of IL-1 induce sleep enhancement similar to the ES responses described here, whereas high doses inhibit sleep (48, 49). EEG desynchronization, which could indicate reduced sleep, is also induced by infusion of IL-1 into the preoptic area of rats (50, 51). In addition, the administration of somnogenic doses of IL-1 to rabbits induces biphasic sleep responses similar to those observed after microbial inoculation (52, 53). The biphasic response to IL-1 and the ability of high doses of IL-1 to suppress sleep are both possible mechanisms by which excessive secretion of IL-1 during a severe or overwhelming microbial challenge such as septic shock could impair sleep.

Despite the likelihood of a role for cytokines in inducing sleep alterations secondary to microbial infections, other factors are also likely to be important. The reduction in slow wave amplitudes, for example, could be mediated via α-melanocyte-stimulating hormone (54), which is the only substance identified to date that reduces EEG slow wave amplitudes. IL-1 induces the release of corticotropin-releasing hormone, which, in turn, induces altered metabolism of propriomelanocorticotropin (55, 56). Both ACTH and α-melanocyte-stimulating hormone are derived from propriomelanocorticotropin, and these hormones could modulate the somnogenic responses to microbial infection. Corticotropin-releasing factor, ACTH, α-melanocyte-stimulating hormone, and, under some circumstances, glucocorticoids all inhibit sleep (6, 54, 57–59). In addition, glucocorticoids have been postulated to attenuate the actions of many immune modulators, including IL-1, and thereby prevent excessive damage to the host as a result of overactivation of inflammatory or immune mechanisms (60–63). Interestingly, immunosuppressive doses of glucocorticoids also attenuate the somno-

genic effects of microbial challenge (6). Higher levels of glucocorticoids occurred in rabbits in the SS group than in rabbits in other groups.

In summary, these data demonstrate that the specific sleep patterns that develop after microbial inoculation reflect the clinical response of the animal. In general, a more favorable prognosis as well as reduced severity of pathophysiological indicators of infectious disease are correlated with sleep patterns characterized by relatively prolonged duration and intensity after infection, whereas a poor prognosis is associated with short periods of enhanced sleep. These observations of prognostic changes in sleep over the course of an infection suggest the exciting possibility of a potential function for sleep. If the hypothesis that sleep promotes recuperation proves to be valid, then a function for sleep will have been identified. Such a discovery would represent an important step in the progress of neurobiological sciences, in that our ability to understand how the brain works is undoubtedly dependent on our ability to understand how sleep benefits the animal.

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