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Photoperiod Alters Duration and Intensity of Non–Rapid Eye Movement Sleep Following Immune Challenge in Siberian Hamsters (*Phodopus sungorus*)

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Sleep is regulated by circadian and homeostatic processes, but can be altered by infectious disease. During infection or exposure to inflammatory stimuli, such as bacterial lipopolysaccharide (LPS), the duration and intensity of non–rapid eye movement sleep (NREMS), as measured by electoencephalogram (EEG) delta waves (.5–4 Hz), increase. These sleep alterations are hypothesized to conserve or redirect energy for immune system activation. Many vertebrates exhibit seasonal changes in immune function and sleep-wake cycle, and photoperiod (day length) serves as a reliable environmental cue. For example, winter is energetically demanding for most animals, and Siberian hamsters (*Phodopus sungorus*) adapted to short winter day lengths display reduced fever after LPS administration to presumably conserve energy. We hypothesized that short days increase the duration and intensity of NREMS after LPS challenge to create additional energy savings, despite evidence to the contrary that high fever is associated with increased NREMS. Male hamsters were housed under long (16 h light (L):8 h dark (D)) or short (8L:16D) day lengths, and chronically implanted with transmitters that recorded EEG and electromyogram (EMG) biopotentials simultaneously or core body temperature. After >10 wks in photoperiod conditions, hamsters received an i.p. injection of LPS or saline (control), and vigilance states (duration and distribution of NREMS, rapid eye movement sleep (REMS), and wakefulness) and EEG delta power spectra (NREMS intensity) were assessed. As expected, LPS treatment increased the duration and intensity of NREMS compared to controls. Hamsters adapted to short photoperiods exhibited cumulatively larger increases in NREMS duration and EEG delta wave amplitude 0–8 h after LPS injection compared to long-day LPS-treated hamsters despite short-day attenuation of fever. These results suggest a seasonal decoupling of LPS-induced fever with sleep to promote energy conservation during predictable energy shortages. Ultimately, the combination of increased sleep and reduced fever could represent a suite of physiological adaptations that increase the probability of surviving winter. (Author correspondence: Noah.Ashley@osumc.edu)

Keywords: Endotoxin, Immune-sleep interaction, Rodent, Seasonality, Somnogen

INTRODUCTION

Sleep is a rapidly reversible state of immobility that is regulated by circadian and homeostatic processes. Under benign conditions, endogenous circadian oscillators, which are primarily entrained by the light-dark cycle, and the duration of prior wakefulness, which homeostatically drives sleep propensity, interact to regulate normal sleep functioning (Borbély, 1982; Borbély & Achermann, 2005). During an infectious challenge or exposure to immunogenic compounds, such as bacterial lipopolysaccharide (LPS), normal sleep-wake cycles are suppressed in favor of non–rapid eye movement sleep (NREMS) (Kreuger et al., 1986; Lancel et al., 1995). This somnogenic effect is primarily mediated by proinflammatory cytokines of the immune system, such as interleukin (IL)-1 and tumor necrosis factor (TNF), that are released from immune cells during an infectious challenge (Krueger & Majde, 1990; Kreuger et al., 1984, 1998; Opp et al., 1991). Central or peripheral administration of these cytokines enhances NREMS and decreases rapid eye movement sleep (REMS) and wakefulness (Kreuger et al., 1984, 1998; Opp, 2005). Although the functional significance of infection-induced sleep remains enigmatic, it has been hypothesized that increased NREMS conserves or redistributes energy for immune system activation and assists in recuperation from infectious disease (Imeri & Opp, 2009; Opp, 2009).

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Many vertebrate species, including humans, exhibit seasonal changes in sleep-wake rhythms as well as immune function, and it has been proposed that these changes optimize energy demands during predictable seasonal fluctuations in the natural environment (Nelson, 2004; Nelson & Demas, 1996). For example, during winter, food is scarce and thermogenic demands are high because ambient temperatures are low. Among humans and other diurnal animals, total sleep time is generally increased and the onset of sleep is advanced during winter to presumably offset the increased energetic demands of longer and colder nights (Jones et al., 2010; Tobler, 1992; Walker et al., 1980; Wehr, 1991). In contrast, nocturnal rodents exhibit a seasonal redistribution of vigilance states, but total sleep duration is not affected (Deboer & Tobler, 1996; Deboer et al., 2000; Franken et al., 1995). Immune function is typically enhanced during winter and this seasonal bolstering is proposed as an adaptation to lessen the impact of disease by anticipating predictable winter stressors (Nelson, 2004; Nelson & Demas, 1996; Nelson et al., 2002). In contrast, inflammatory responses and fever are reduced during winter (Bilbo et al., 2002b; Bilbo & Nelson, 2002) because the high energetic costs of these responses likely compete with other physiological systems important for winter survival.

Changing day length (photoperiod) is a reliable environmental cue that many animals use to forecast seasonal energy deficits. Outside of the tropics, most vertebrates (excluding humans) breed on a seasonal basis. Among small rodents, breeding is normally curtailed during winter in favor of survival mechanisms, such as thermoregulation. For example, during winter or exposure to short day lengths in captivity, Siberian hamsters (*Phodopus sungorus*) grow thicker pelage and reduce body mass by ~25%, and these adaptations result in energy savings by preventing heat loss and reducing daily fuel requirements, respectively (Heldmaier & Steinlechner, 1981). These photoperiodically regulated changes in reproduction and thermoregulation persist despite mild temperatures and *Ad libitum* food in captive conditions. Short days (SD), as compared to long days (LD), also diminish fever and anorexia following LPS administration and decrease production of proinflammatory cytokines (Bilbo et al., 2002b). Whether increased sleep following immune challenge is similarly attenuated upon exposure to short days is unknown.

The aim of this present study was to determine whether short days alter LPS-induced sleep in Siberian hamsters. Two competing hypotheses are proposed to explain how photoperiod might influence sleep after immune challenge. Firstly, if the neurobiological and humoral pathways that regulate the seasonal suppression of inflammation and fever in SD hamsters are similar or shared with sleep-promoting areas of the brain, then it is predicted that short day lengths will diminish NREMS enhancement following immune challenge. It is widely recognized that sleep and thermoregulation are interconnected and regulated by overlapping neural circuits in the brain (Parmeggiani, 2003). Secondly, and alternatively, if energy conservation is important during winter, then SD hamsters are predicted to increase LPS-induced NREMS amount relative to LD hamsters to save energy. This hypothesis is corroborated by previous studies reporting decreased energy expenditure during NREMS bouts relative to REMS or wakefulness (Berger & Phillips, 1995; Brebbia & Altshuler, 1965). Evidence supporting this alternative hypothesis would suggest a seasonal dissociation of mechanisms governing the somnogenic and pyrogenic effects of antigenic challenge. In addition, electroencephalographic (EEG) delta power (.5–4.0 Hz) of NREMS was assessed following LPS injection to measure intensity, or depth, of NREMS (Borbély, 1982; Borbély & Achermann, 2005). An increase of NREMS EEG delta power is predicted to conserve energy by decreasing the probability of awakening because increased delta wave amplitude is associated with higher thresholds of arousal (Neckelmann & Ursin, 1993), and transitions from NREMS to wakefulness increase energetic costs (Berger & Phillips, 1995; Bonnett et al., 1991; Brebbia & Altshuler, 1965; Jung et al., 2011).

MATERIALS AND METHODS

Animals and Light/Dark Schedule

Male Siberian hamsters (N = 20) were born in a reverse long (16 h light/8 h dark) photoperiod in a colony room (lights-on at 22:00 h) at the Ohio State University. After weaning at 21–24 d of age, hamsters were singly housed in polypropylene cages (27.8 × 7.5 × 13 cm) at an ambient temperature of 21°C ± 2°C and randomly assigned to reverse short (8 h dark/16 h light; lights-on at 06:00 h) photoperiod in a separate room or to remain in LD. Hamsters were provided cotton batting for nesting material, food (Harlan Teklad 8640 rodent diet; Indianapolis, IN), and filtered tap water *ad libitum* throughout the experiment. This study was conducted under approval of The Ohio State University Institutional Animal Care Committee and procedures followed the National Institutes of Health *Guide for the Use and Care of Laboratory Animals* and international ethical standards (Portaluppi et al., 2010).

Surgical Methods

Following 9–10 wks of photoperiod conditions, hamsters were surgically implanted with PhysioTel F20-EET biotelemetry transmitters (Data Sciences International [DSI], St. Paul, MN) according to the manufacturer's protocol (DSI EET Device Surgery Manual). This transmitter simultaneously measures EEG and electromyogram (EMG) biopotentials. Hamsters were deeply anesthetized with isoflurane (5% induction, 1.5% maintenance) and immobilized in a stereotaxic apparatus. A midline incision from the posterior margin of the eyes to midway between the scapulae was made, and the skull

was exposed and cleaned. Two stainless steel screws (00-96 × 1/16; Plastics One, Roanoke, VA) served as cortical electrodes and were inserted through the skull to make contact with the dura membrane. The first screw was positioned 1 mm lateral to the sagittal suture and 1 mm anterior to bregma; the second screw was placed contralaterally 2 mm from the sagittal suture and 2 mm posterior to bregma. The transmitter was inserted into a subcutaneous pocket along the back with biopotential leads oriented cranially. One set of leads was attached to screws and secured with dental acrylic; the other set was directly inserted into the cervical trapezius muscles for measurement of EMG. Supplemental warmth and analgesia (buprenorphine) were administered after surgery. Animals were allowed to recover for >10 d before starting the experiment.

Experimental Protocol

LD and SD hamsters were moved into light-controlled, sound-attenuating ventilated cabinets that replicated lighting in original rooms to accommodate analysis of sleep. In these ventilated cabinets, ambient temperature was maintained at 24°C ± 2°C. Cages were placed on receivers boards (RPC-1; DSI) that relay telemetered data from the transmitter to a data exchange matrix (DSI) and a personal computer running Dataquest A.R.T. software (version 4.1). EEG and EMG traces were measured for 48 h (starting at lights-off at 14:00 h) to evaluate baseline sleep.

Following these recordings, hamsters received an intraperitoneal (i.p.) injection (.1 cc) of either bacterial LPS (25 μg; serotype 026:B6; Sigma, St. Louis, MO) or .9% saline (SAL) just before lights-off and were returned to home cages. Biopotentials were recorded up to 24 h to assess the effect of LPS upon sleep. After 8 d of additional recovery, the experimental protocol was repeated, but injection type (LPS or SAL) was switched for each hamster using a counterbalanced design. Animals were then euthanized and weighed (with transmitter still implanted). Paired testes, epididymides, seminal vesicles, and inguinal adipose tissue were also removed and weighed. Two hamsters were considered reproductively nonresponsive to SD because paired testes mass was greater than 2 standard deviations below the average paired testes mass for LD hamsters. Two LD hamsters were removed from the experiment for health-related reasons. After excluding these hamsters, 8 SD and 8 LD animals remained in the study.

Fever responses to LPS were assessed in another cohort of hamsters (LD, N = 6; SD, N = 6; after >10 wks in photoperiod conditions) to examine whether higher ambient temperatures in the ventilated cabinets altered the SD attenuation of fever that was previously reported at room temperature (21°C ± 2°C; Bilbo et al., 2002b). To measure core body temperature (CBT), LD and SD hamsters were deeply anesthetized as described above and then implanted i.p. with battery-free transponders (Mini-Mitter, Bend, OR) that measure CBT. Cages were placed on TR-4000 receiver boards, which generate power for the transponders to emit temperature frequencies. These data were then converted to temperature values using calibration curves unique to each transponder and binned every 15 min by VitalView software (Mini-Mitter) running on a personal computer. Hamsters were kept in identical conditions (ventilated cabinets at 24°C ± 2°C) as described above. Hamsters were injected i.p. with either LPS (same dose as above) or SAL just before lights-off (14:00 h) and CBT was measured for 18 h. One week later, the experiment was repeated, but treatment types were switched using a counterbalanced approach.

Sleep Analysis

EEG data were filtered (high pass, .3 Hz; low pass, 35 Hz), digitized (500 Hz), partitioned into 10-s epochs, and stored on a hard drive. EMG data were digitized (500 Hz), full-wave rectified, and integrated as one value within each 10-s epoch. Data were visually inspected, and epochs containing artifacts were removed from further analysis. Each 10-s epoch was identified as wakefulness (high EMG amplitude, low EEG amplitude), REMS (low EMG amplitude, low EEG amplitude), or NREMS (low EMG amplitude, high EEG amplitude) using an algorithm that exhibits >90% concordance with visual scoring in mice and rats (Benington et al., 1994; Veasey et al., 2000) and has been previously utilized in Siberian hamsters (Larkin et al., 2004). The amount of time spent in each vigilance state was calculated on an hourly basis (expressed as a percentage of artifact-free recording time). To assess NREM intensity, EEG data from each epoch were subjected to power analysis (fast Fourier transforms) in the delta (.5–4.0 Hz) frequency range to evaluate slow-wave activity (SWA), and then averaged into 1-h bins.

Statistics

Two-tailed *t* tests were used to assess photoperiodic changes in body, reproductive organ, and fat pad masses. To evaluate the effect of photoperiod (SD vs. LD) upon baseline sleep parameters, separate one-way, repeated-measures analysis of variance (rmANOVA) tests were conducted on the data of the first and second 24-h baseline periods, respectively. Photoperiod was the main effect, time (h) was the repeated measure, and duration (%) of wakefulness, NREMS, or REMS were dependent variables. Angular transformation successfully normalized the vigilance state percentages. If significant effects or interactions involving photoperiod were detected, then unpaired *t* tests (two-tailed) were conducted across 8-h time blocks to specify photoperiodic differences relative to changes in the light-dark cycle. For these comparisons, significance levels were adjusted according to sequential Bonferonni correction (Sokal & Rohlf, 1995). The effect of photoperiod upon the cumulative time spent in vigilance states during dark, light, and 24-h periods was assessed using unpaired *t* tests. The effect of the light-dark cycle upon vigilance states was evaluated using two-tailed paired *t* tests.

To determine whether LPS treatment affected sleep and CBT, paired *t* tests (with sequential Bonferonni correction) were employed to assess responses of SD and LD hamsters to LPS and SAL injection, since animals served as their own controls. Photoperiodic effects upon sleep and CBT following LPS or SAL injection were evaluated using one-way rmANOVAs as described above, except that dependent variables (vigilance states, slow-wave delta power) were expressed as a percentage change relative to vehicle (SAL) injection. CBT data were expressed as the absolute difference relative to vehicle. These comparisons take into account within-individual and circadian variations. There were no significant effects of injection order (1st or 2nd round) using rmANOVA (all $p > .15$). Thus, injection order as a variable was collapsed within treatment groups. If significant effects or interactions involving photoperiod were detected, then *t* tests (with sequential Bonferonni correction) were used to test for photoperiodic differences across 6- and 8-h time blocks for CBT and sleep, respectively. Fever duration of LPS-injected hamsters was calculated by determining the duration that body temperature (in 15-min intervals) was above the active phase baseline (following vehicle injection) for each individual. Unpaired two-tailed *t* tests were used to determine the effect of photoperiod upon fever duration. Unless otherwise noted, significance was designated at $p < .05$.

RESULTS

Body and Reproductive Organ Mass

Body, reproductive organ, and fat pad masses were significantly lower ($p < .01$ for all) in SD hamsters (N = 8) compared to LD hamsters (N = 8; Figure 1).

Baseline Sleep

Photoperiod significantly altered the time spent in wakefulness ($p < .05$) and in NREMS ($p = .02$) during the first 24-h baseline recording. Specifically, LD hamsters spent significantly less time awake ($p = .004$, corrected $\alpha = .017$) and more time in NREMS ($p = .003$, corrected $\alpha = .017$) within an 8-h time block starting at 22:00 h (lights-on for LD hamsters) compared to SD hamsters (lights remained off; Figure 2A, B). For the second 24-h baseline period, photoperiodic differences in wakefulness and NREMS duration were no longer apparent ($p > .45$; Figure 2A, B). Photoperiod did not significantly influence the amount of REMS during the first ($p = .052$) and second ($p = .07$) 24-h baseline periods (Figure 2C). Cumulatively, SD hamsters spent proportionately less time in NREMS (38%) within the first 24-h baseline period relative to LD hamsters (26%; Table 1). During the dark phase, SD hamsters spent a larger percentage of time in REMS relative to LD hamsters, and this effect was consistent for the two baseline 24-h periods (Table 1). Vigilance states were also distributed differently over the light-dark cycle. The dark (active) phase increased NREMS and increased wakefulness compared to the light (inactive) period for SD hamsters during both 24-periods and for LD hamsters during the second 24-h period. The light-dark cycle did not significantly alter REMS amount (Table 1).

LPS-Induced Sleep

LPS treatment significantly reduced wakefulness in SD and LD hamsters (all $p < .0001$; corrected $\alpha = .017$; Figure 3A) 0–8 h after injection compared to vehicle. Decreases in wakefulness after LPS coincided with significant increases in NREMS (all $p < .0002$; corrected $\alpha = .017$; Figure 3B). There was a trend for LPS treatment

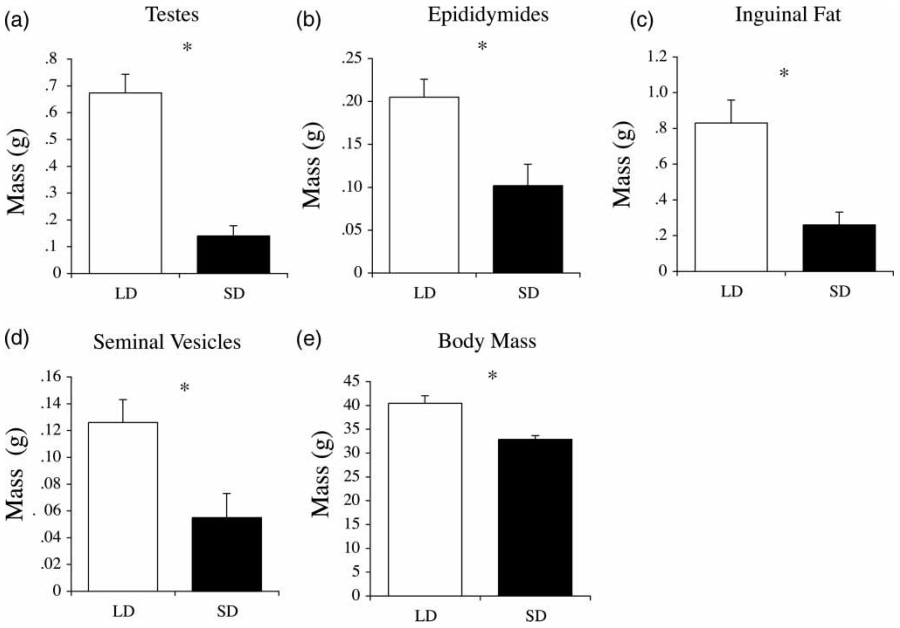

FIGURE 1. Mean (± SEM) reproductive tissue and body masses (g): (A) paired testes (B) epididymides (C), inguinal fat pad mass, (D) seminal vesicles, and (E) body mass (including mass of implanted transmitter). Asterisk denotes $p < .05$.

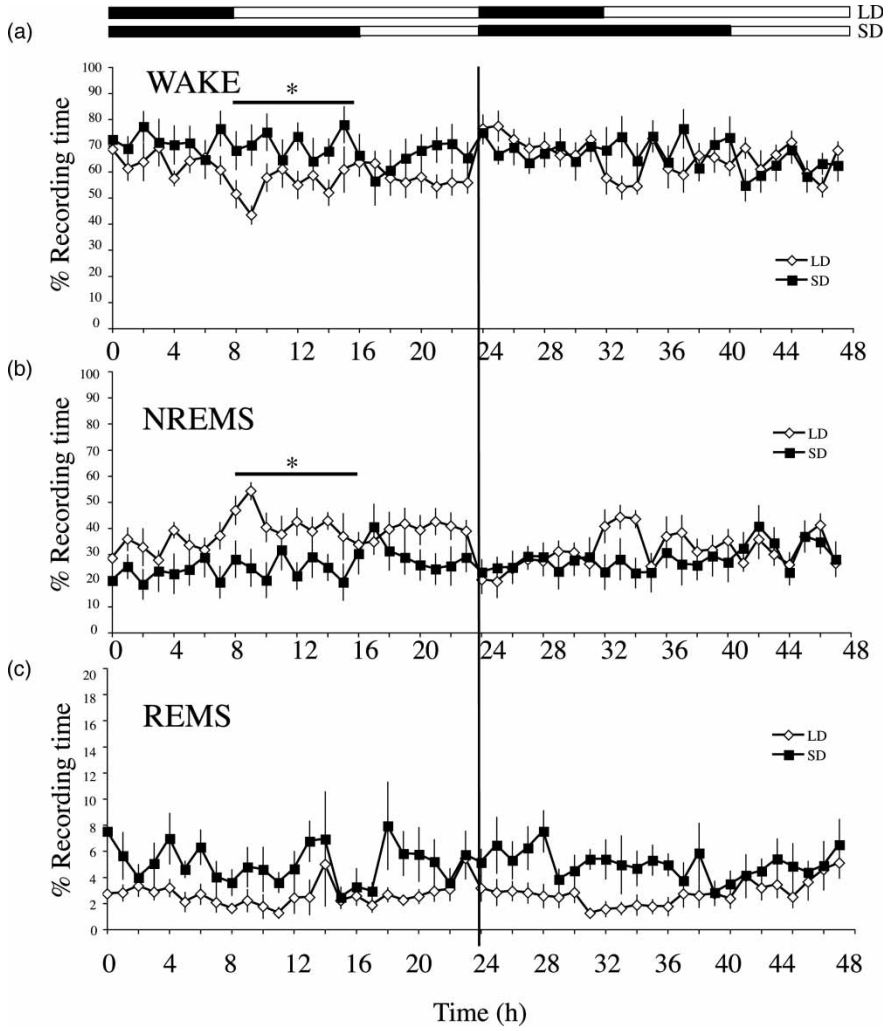

FIGURE 2. Mean (± SEM) percentage of recording time spent in (A) wakefulness, (B) NREMS, and (C) REMS over a 48-h baseline period. Asterisk indicates significant difference between photoperiod treatments for 8-h time blocks. White and black bars above denote the light and dark portion of the light-dark cycle, respectively.

Table 1. Effect of photoperiod upon daily sleep-wake cycles of Siberian hamsters over two consecutive 24-h periods.

	% recording time			
	First 24-h period		Second 24-h period	
	LD	SD	LD	SD
NREMS				
Dark	33.41 ± 3.04	23.98 ± 3.89	26.07 ± 2.82	26.40 ± 3.29
Light	40.79 ± 2.88	29.54 ± 4.98*	34.47 ± 3.47*	32.24 ± 4.07*
24-h	**38.33 ± 2.42**	**25.83 ± 4.19**	31.67 ± 3.14	28.35 ± 3.46
REMS				
Dark	**2.73 ± .56**	**5.10 ± .96**	**2.61 ± .64**	**5.14 ± .89**
Light	2.65 ± .73	5.04 ± 1.34	2.85 ± .82	4.78 ± 1.20
24-h	2.68 ± .51	5.08 ± 1.07	2.77 ± .69	5.01 ± .95
WAKE				
Dark	63.85 ± 3.34	72.55 ± 4.13	71.33 ± 2.39	68.53 ± 2.89
Light	56.58 ± 2.58	62.68 ± 3.47*	62.68 ± 3.16*	62.68 ± 3.47*
24-h	59.00 ± 2.21	69.09 ± 4.19	65.56 ± 2.83	66.58 ± 2.97

Hamsters were adapted to long days (LD; 16L:8D) or short days (SD; 8L:16D) for >10 weeks before measuring sleep. Bold font indicates a significant effect of photoperiod ($p < .05$) using unpaired t tests. An asterisk indicates $p < .05$ relative to the dark period using paired t tests. Values represent the mean ± SEM.
LD = long days; SD = short days; NREMS = non–rapid eye movement sleep; REMS = rapid eye movement sleep; WAKE = wakefulness.

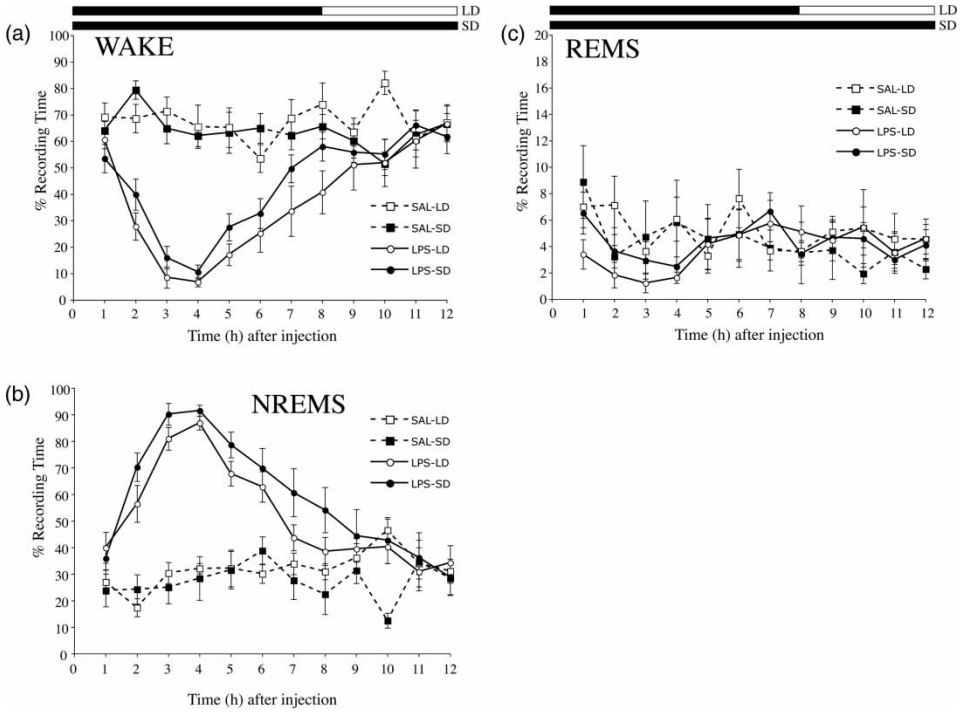

FIGURE 3. Mean (± SEM) percentage of recording time spent in (A) wakefulness, (B) NREMS, and (C) REMS from 0 to 12 h following LPS or saline (SAL) injection in hamsters exposed to short days (SD) or long days (LD). Hamsters were injected at the beginning of the dark cycle (14:00 h). White and black bars above denote the light and dark portion of the light-dark cycle, respectively.

to reduce REMS duration in SD hamsters ($p = .047$, corrected $\alpha = .017$) relative to vehicle, but not in LD hamsters ($p = .68$; Figure 3C). Vigilance states during other 8-h time blocks (8–16 h and 16–24 h post injection) were not significantly affected by LPS treatment (all $p > .12$; data not shown).

Relative to values obtained after administration of vehicle, SD hamsters injected with LPS exhibited a significantly larger percentage increase in NREMS 0–8 h post injection compared to LD hamsters treated with LPS ($p < .05$), but neither wakefulness ($p = .12$) nor REMS ($p = .32$) duration was significantly affected by photoperiod (Figure 4). LPS increased NREM delta power of SD and LD hamsters relative to vehicle 0–8 h post injection (all $p < .013$). SD animals treated with LPS exhibited larger percentage increases in NREMS delta wave amplitude relative to LPS-treated LD hamsters (photoperiod × time interaction: $p < .05$). These differences were most apparent 3 h after LPS challenge (Figure 5).

Fever

LPS significantly increased CBT averaged from 0 to 6 h after injection relative to vehicle injection in SD and LD

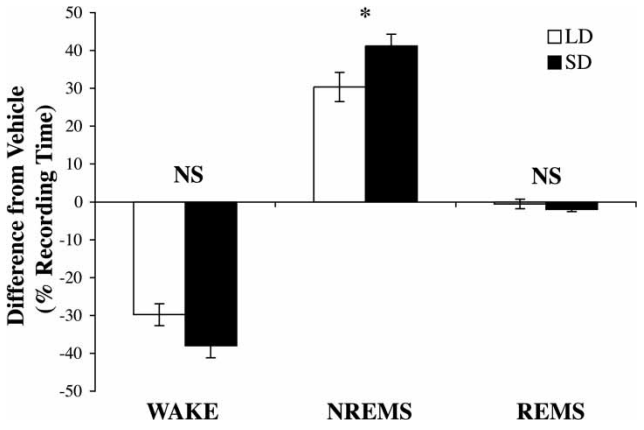

FIGURE 4. Effect of photoperiod upon cumulative measures of wakefulness (WAKE), NREMS, and REMS from 0 to 8 h after LPS injection. Values are expressed as the mean (± SEM) percentage difference relative to values obtained after vehicle injection in the same animal. Asterisk denotes a significant difference between photoperiod ($p < .05$; NS denotes $p > .05$).

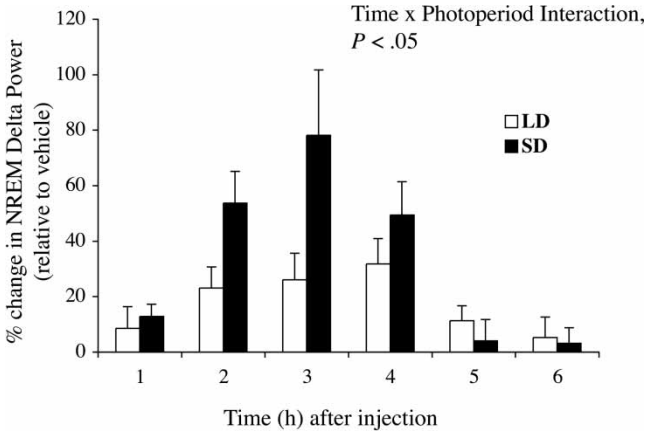

FIGURE 5. Effect of photoperiod upon EEG delta power 0–6 h following LPS injection. Values are expressed as the mean (± SEM) percentage difference relative to values obtained after vehicle injection in the same animal.

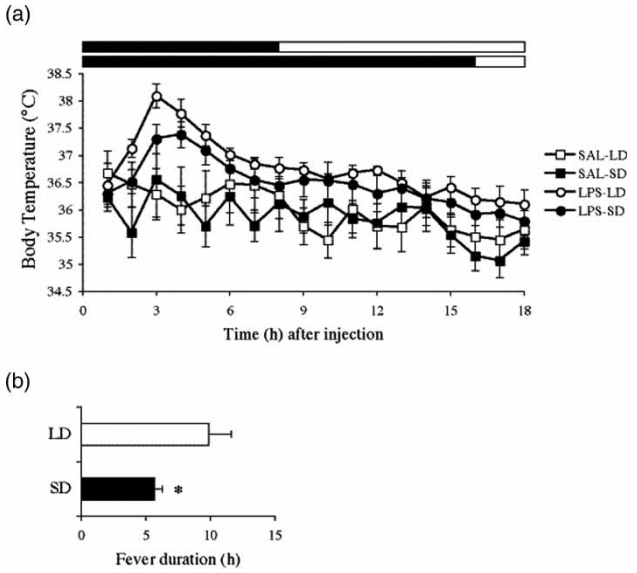

FIGURE 6. (A) Effect of LPS or saline (SAL) injection upon hourly core body temperature (mean ± SEM) between LD and SD hamsters. White and black bars above denote the light and dark portion of the light-dark cycle, respectively. (B) Effect of LPS upon fever duration (h; mean ± SEM) between LD and SD hamsters. Asterisk denotes a significant difference between photoperiod ($p < .05$).

hamsters (paired t test; all $p < .016$, corrected $\alpha = .017$), but not from 6 to 12 h or from 12 to 18 h post injection (all $p > .07$). Among LPS-injected hamsters, CBT changes relative to vehicle were significantly altered by a photoperiod × time interaction (rmANOVA, $p < .0001$), although no photoperiodic differences were detected when these changes were averaged into 6-h blocks (all $p > .58$; Figure 6A). Duration of fever was significantly decreased in SD hamsters compared to LD hamsters ($p = .03$; Figure 6B).

DISCUSSION

It has been proposed that sleep is a physiological adaptation that reduces energy use during periods when activity is not beneficial (Siegel, 2009). This viewpoint has been extended to explain the occurrence of increased NREMS during the acute response to infection (Imeri & Opp, 2009). Our study indicates that short day lengths increase the duration and intensity of NREMS induced by peripheral administration of LPS during the dark (active) phase. These results support the alternative hypothesis that a diminished energy budget during winter alters physiological responses to LPS, a potent somnogen and pryogen. Specifically, increased duration and intensity of non-REM sleep likely conserves energy by preventing activity costs associated with wakefulness (Imeri & Opp, 2009).

Seasonal Decoupling of Infection-Induced Sleep From Fever?

Previous studies have consistently reported that fever duration is reduced in Siberian hamsters housed under short day lengths (Bilbo et al., 2002b; Prendergast, 2008; Prendergast et al., 2008; Wen & Prendergast, 2007; Wen et al., 2007). Importantly, these studies were conducted at room temperature (20°C ± .5°C or 21°C ± 2°C). Because our experiment was carried out in warmer ambient temperatures (24°C ± 2°C), it is possible that thermoregulatory demands could have been altered such that short-day attenuation of fever would no longer be apparent. However, our data indicate that this attenuation is preserved. SD hamsters exhibited a significant reduction in fever duration after LPS injection compared to LD hamsters, which is consistent with previous studies. These data are not surprising considering that the thermoneutral zone of Siberian hamsters falls within 20–30°C (Heldmaier & Steinlechner, 1981). Within this zone, hamsters do not increase metabolic rate to regulate body temperature. It would be intriguing to measure fever responses in hamsters exposed to ambient temperatures below their thermoneutral zone to assess whether increased energy expenditure from cold exposure leads to a more pronounced enhancement of NREMS and attenuation of fever in SD hamsters.

SD attenuation of fever is likely a result of selective pressures that favor strategies to minimize energy expenditure during seasonal energy deficits (Bilbo et al., 2002b; Nelson, 2004). For example, the energetic costs of activating fever are considerable, requiring a ~13% increase in metabolic rate for every 1°C rise in body temperature (Kluger et al., 1998). These costs likely create trade-offs among competing physiological systems such that the generation of a sustained fever response is constrained. We hypothesize that the energy savings accrued from increased NREMS buffers some of the costs of generating fever, but they are not recouped completely, because fever duration is still reduced upon exposure to short photoperiod (this study; Bilbo et al., 2002b). Studies that experimentally block NREMS using pharmacological or behavioral methods and concomitantly measure energy expenditure could potentially address this possibility. Alternatively, Siberian hamsters display enhanced cell-mediated immune function in short day lengths (Bilbo et al., 2002a), and immunoenhancement could be involved with fever attenuation rather than energy balance, per se, although this possibility has not been tested.

Our data suggest a photoperiod-mediated decoupling of LPS-induced fever with sleep to promote energy conservation during winter. This result conflicts with observations that sleep and CBT are inextricably interconnected. For example, there are tight associations between the circadian rhythm of CBT and sleep, and small deviations in brain temperature can alter sleep states (Parmeggiani, 2003). On a mechanistic level, several areas of the brain, namely the preoptic area and basal forebrain, regulate sleep and thermoregulation. Neurons in the ventrolateral preoptic area (VLPO) and ventromedial preoptic nucleus (VmPO) exhibit Fos immunoreactivity after sleep (Dentico et al., 2009; Sherin et al., 1996), and electrophysiological studies

show that sleep-active neurons are abundant in these regions (Suntsova et al., 2002; Szymusiak et al., 1998). The preoptic area is also important for thermoregulation and fever, and increased brain temperature can promote NREMS (McGinty et al., 1994). Given this information, it seems likely that diminished fever in SD-adapted hamsters should lead to a corresponding decline in NREMS rather than an increase. Nonetheless, there is anatomical and pharmacological evidence to suggest that the control of fever and sleep are closely linked but separate processes (Kreuger & Takahashi, 1997). First, regulation of sleep and thermoregulation appears to occur in different regions within the preoptic area. Highly accurate lesions of the VLPO promote insomnia, but do not alter CBT or its circadian variation (Lu et al., 2000). Moreover, lesions of the VmPO increase the amplitude of CBT rhythms, whereas sleep is unaffected (Lu et al., 2000). Second, the neurobiological pathways that regulate sleep and fever are not identical. Antipyretics block IL-1-induced fever, but not sleep (Kreuger et al., 1984). Conversely, nitric oxide synthase inhibitors decrease IL-1–induced sleep, but not fever (Kapás et al., 1994). Furthermore, IL-6, a cytokine produced during the acute phase response, activates fever but does not alter sleep. Microinjection of IL-1 into the preoptic area induces fever but not sleep (Walter et al., 1989). Lastly, the time course of fever is different from those of sleep responses to LPS (Kreuger et al., 1998). Taken together, these studies imply that selection could act upon sleep and fever differentially to produce a decoupled response.

Enhancement of NREMS following LPS challenge was also associated with decreased wakefulness and a strong tendency for reduced REMS. During normal sleep, transitions from NREMS to REMS are generally accompanied by an increase in brain and core temperatures (Obál et al., 1985; Parmeggiani, 2003). However, during fever, REMS is generally inhibited, which suggests active suppression. Because REMS inhibits shivering (Glotzbach & Heller, 1976), it has been proposed that suppression of REMS during infection promotes shivering thermogenesis for the generation of fever (Imeri & Opp, 2009).

Our results indicate that LPS-induced sleep is modulated by photoperiod. Melatonin, an indole amine hormone produced and released from the pineal gland primarily at night, could regulate such photoperiodic changes. Exposure to SD increases the duration of elevated blood melatonin compared to exposure to LD, regardless of whether an animal is diurnal or nocturnal (Arendt, 2006; Reiter, 1993). Many of the seasonal changes in immune function and sleep have been attributed to the immunoenhancing and somnogenic effects of melatonin (Maestroni & Conti, 1993; Nelson, 2004; Nelson & Demas, 1996; Nelson et al., 2002; Wehr, 1991), respectively, although studies examining the effect of melatonin upon sleep have produced conflicting results (for review, see van den Heuvel et al., 2005). In Siberian hamsters, chronic melatonin implantation abolishes the circadian rhythm of light-dark cycles and suppresses REMS, but it fails to recapitulate SD sleep patterns (Deboer & Tobler, 1997). However, daily injections of melatonin in LD (breeding) Siberian hamsters induces gonadal regression and reduces the duration of fever compared to saline-injected controls (Bilbo & Nelson, 2002). Future studies are necessary to determine whether short-duration melatonin signals enhance sleep after immune challenge and whether melatonin can differentially influence areas in the brain important for thermoregulation and NREMS.

Photoperiodic Regulation of Baseline Sleep

The effect of photoperiod upon baseline sleep was also assessed, which has been studied previously in this species (Deboer & Tobler, 1996; Deboer et al., 2000). It is unclear why considerable differences between the first and second 24-h recording periods occurred, but we speculate that sleep was altered because hamsters had recently been moved to ventilated, sound-attenuating cabinets, and this novel environment with slightly higher ambient temperature than room temperature could have influenced sleep architecture. During the first 24-h period, LD-adapted hamsters decreased wakefulness and increased REMS at the start of their light (inactive) phase (at 22:00 h) compared to SD-adapted hamsters, which remained in the dark phase until 18:00 h. These results are consistent with increased wakefulness that occurs at night compared to daytime in Siberian hamsters (Deboer & Tobler, 1996). By the second day, however, these photoperiodic differences in sleep-wake rhythms were no longer detected. The only consistent photoperiodic difference that occurred over the two consecutive recording days was that SD hamsters spent nearly twice as long in REMS during the dark period compared to LD hamsters. The effect has been described previously in Siberian hamsters (Deboer & Tobler, 1996), although its functional significance is unclear.

Despite these similarities, there were notable differences in total sleep time and circadian regulation of sleep-wake cycles compared to previous investigations that examined photoperiodic effects upon sleep (Deboer & Tobler, 1996; Deboer et al., 2000; Kreuger et al., 1984; Larkin et al., 2004). In this study, total sleep time was 41% and 31% for hamsters adapted to LD and SD photoperiods, respectively, compared to 62% and 59% in a previous study (Deboer & Tobler, 1996). Furthermore, the circadian distribution of NREMS, REMS, and wakefulness was blunted relative to other studies (Deboer & Tobler, 1996; Larkin et al., 2004). For these latter studies, assessment of sleep occurred in restrained hamsters connected to recording cables, whereas hamsters in this study were unrestrained because sleep was recorded wirelessly using radiotransmitters. Given these differences, it is predicted that measurement of unrestrained hamsters would provide a more accurate representation of sleep. Nevertheless, it is also equally probable that transmitter implantation directly affected behavior and sleep,

although animals were provided >10 d to recover from surgery. Despite these discrepancies, the sleep data generated were consistent with studies demonstrating NREMS enhancement following LPS challenge (Kreuger et al., 1984, 1986; Lancel et al., 1995).

Conclusions

This study provides evidence that short days enhance the duration and intensity of NREMS sleep following antigenic challenge by LPS administration. The combination of increased sleep and reduced fever could represent a suite of physiological adaptations that increase the probability of surviving winter through energy conservation. Although the SD enhancement of LPS-induced NREMS has been identified, the neurobiological mechanisms that regulate such changes are not known, as well as the contribution of various humoral factors, such as melatonin and reproductive hormones, which vary on a seasonal basis. Siberian hamsters, which exhibit pronounced seasonal changes in physiology and behavior, will likely serve as an ideal model species in the future to investigate the proximate mechanisms underlying photoperiodic regulation of baseline and infection-induced sleep. The simultaneous measurement of brain temperature and CBT with EMG/EEG biopotentials will help elucidate the apparent decoupling of sleep from febrile responses in SD hamsters. Lastly, the effect of immune challenge upon sleep has important implications for a variety of inflammatory, infectious, and cardiovascular diseases (Lorton et al., 2006). Understanding how photoperiod affects cytokine-mediated sleep could elucidate new treatment options for patients suffering from dysregulated sleep and fatigue as a result of chronic inflammatory disease, cancer, or seasonal affective disorders.

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