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RESEARCH ARTICLE

Sleep deprivation attenuates endotoxin-induced cytokine gene expression independent of day length and circulating cortisol in male Siberian hamsters (*Phodopus sungorus*)

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SUMMARY

Sleep is restorative, whereas reduced sleep leads to negative health outcomes, such as increased susceptibility to disease. Sleep deprivation tends to attenuate inflammatory responses triggered by infection or exposure to endotoxin, such as bacterial lipopolysaccharide (LPS). Previous studies have demonstrated that Siberian hamsters (*Phodopus sungorus*), photoperiodic rodents, attenuate LPS-induced fever, sickness behavior and upstream pro-inflammatory gene expression when adapted to short day lengths. Here, we tested whether manipulation of photoperiod alters the suppressive effects of sleep deprivation upon cytokine gene expression after LPS challenge. Male Siberian hamsters were adapted to long (16 h:8 h light:dark) or short (8 h:16 h light:dark) photoperiods for >10 weeks, and were deprived of sleep for 24 h using the multiple platform method or remained in their home cage. Hamsters received an intraperitoneal injection of LPS or saline (control) 18 h after starting the protocol, and were killed 6 h later. LPS increased liver and hypothalamic interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF) gene expression compared with vehicle. Among LPS-challenged hamsters, sleep deprivation reduced IL-1 mRNA levels in liver and hypothalamus, but not TNF. IL-1 attenuation was independent of circulating baseline cortisol, which did not increase after sleep deprivation. Conversely, photoperiod altered baseline cortisol, but not pro-inflammatory gene expression in sleep-deprived hamsters. These results suggest that neither photoperiod nor glucocorticoids influence the suppressive effect of sleep deprivation upon LPS-induced inflammation.

Key words: cortisol, cytokine, endotoxin, hypothalamus, inflammation, interleukin-1, photoperiod, Siberian hamster, sleep deprivation.

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INTRODUCTION

Sleep is generally viewed as a restorative process, and its curtailment or suspension induces cognitive, metabolic, immunological and inflammatory impairments in humans (Simpson and Dinges, 2007; Mullington et al., 2009; Faraut et al., 2012) as well as other animal species [e.g. bees (Beyaert et al., 2012), songbirds (Jones et al., 2010), rodents (Zager et al., 2007; Zager et al., 2012)]. A growing body of experimental and epidemiological studies has demonstrated that sleep loss induces alterations in the immune system that predispose individuals towards disease (Faraut et al., 2012).

Although the underlying mechanisms that link sleep loss to pathological outcomes are not fully understood, there is mounting evidence that pro-inflammatory mediators are involved (Simpson and Dinges, 2007; Mullington et al., 2009; Faraut et al., 2012). Reduced or disordered sleep creates a low-grade inflammatory environment that involves local and peripheral release of pro-inflammatory cytokines, such as interleukin-1 (IL-1), IL-6 and tumor necrosis factor-alpha (TNF) (Irwin et al., 1996; Frey et al., 2007; van Leeuwen et al., 2009). Even a single night of partial sleep loss can induce a rapid increase in nuclear-factor-kappa B activity, the transcription factor that promotes pro-inflammatory gene expression (Irwin et al., 2008; Irwin et al., 2010). Alternatively, during an infection or inflammatory challenge, such as bacterial lipopolysaccharide (LPS) exposure, sleep deprivation attenuates cellular immunity (Zager et al., 2012), alters sickness behavior (Zager et al., 2009) and suppresses pro-inflammatory mRNA expression in the periphery and brain (Weil et al., 2009). A common feature of sleep loss is activation of the hypothalamic-pituitary-adrenal axis (Meerlo et al., 2002), which leads to a moderate and transient elevation in circulating glucocorticoids. However, it remains unspecified whether changes in immune function are directly mediated by these immunomodulatory hormones (Redwine et al., 2000; Bryant et al., 2004).

It is well documented that sleep and immune function vary on a seasonal basis (Wehr, 1991; Nelson and Demas, 1996). Animals inhabiting non-tropical regions use photoperiod (day length) as a reliable and predictive environmental cue to synchronize physiology and behavior with daily and annual geophysical cycles. For example, most vertebrates, including humans, exhibit fluctuations in the prevalence of particular diseases over the year (Nelson, 2004). These seasonal trends are not governed solely by pathogen dynamics because seasonal changes in host immune function can be recapitulated in captivity by manipulating photoperiod (Nelson and Demas, 1996; Nelson et al., 2002; Nelson, 2004; Martin et al., 2008).

This modulation typically involves immunoenhancement upon exposure to short days to presumably anticipate energetic stressors and increased susceptibility to disease during winter. By contrast, inflammatory and febrile responses are sufficiently expensive to produce that their suppression is favored during the energetically demanding conditions of winter (Bilbo et al., 2002). Similarly, the duration, distribution, intensity and overall quality of sleep is affected by season, with total sleep time increasing, sleep onset advancing and/or the amplitude of electroencephalographic (EEG) waves decreasing in winter compared with summer (Wehr, 1991; Deboer and Tobler, 1996; Jones et al., 2010).

Less understood is the effect of photoperiod upon immune–sleep interactions. A recent study demonstrated that photoperiod alters how sleep is affected by immune challenge in Siberian hamsters [*Phodopus sungorus* (Pallas 1773)], with short days increasing the duration and intensity of slow-wave sleep after exposure to LPS (Ashley et al., 2012). However, to our knowledge, the reciprocal interaction, the effect of photoperiod on inflammatory responses under conditions of sleep deprivation, has not been examined.

The aim of this study was to determine whether photoperiod alters the suppressive effect of sleep deprivation on endotoxin-induced inflammation. Specifically, pro-inflammatory cytokine gene expression was measured in the periphery and brain following intraperitoneal injection of LPS. Past studies in Siberian hamsters have demonstrated that short day lengths attenuate febrile and behavioral responses to peripheral or central injection of LPS (Bilbo et al., 2002; Fonken et al., 2012), and IL-1 and TNF mRNA expression in the hypothalamus is correspondingly blunted (Pyter et al., 2005). In addition, short days decrease EEG power density (a measure of intensity), but do not affect total duration of sleep (Deboer and Tobler, 1996). Given the photoperiodic difference in EEG intensity, it is conceivable that short-day-adapted hamsters are less susceptible to the effects of sleep deprivation compared with long-day-adapted hamsters. Thus, we predicted that short days would diminish the suppressive effects of sleep deprivation upon pro-inflammatory gene expression after LPS challenge.

MATERIALS AND METHODS

Animals

Ninety male adult Siberian hamsters were used from our breeding colony. After weaning at 21–24 days of age, males were housed individually in polypropylene cages (27.8×7.5×13 cm) at an ambient temperature of 21±2°C, which is within the thermoneutral zone of this species (Heldmaier and Steinlechner, 1981). Hamsters were then assigned to either a reverse long photoperiod (LP; 16 h:8 h light:dark, lights on at 22:00 h; N=40) or a reverse short photoperiod (SP; 8 h:16 h light:dark, lights on at 06:00 h; N=50) for >10 weeks. Twenty percent of hamsters adapted to SP (N=10) were considered reproductively non-responsive to photoperiod because testes mass was greater than 2 standard deviations below the average paired testes mass of hamsters exposed to LP. This value ranges between 15 and 30% in previous studies (Puchalski and Lynch, 1986; Nelson, 1987) and probably reflects the current genetic make-up of our colony. These non-responding hamsters were removed from the study. Food (Teklad 8640 rodent diet, Harlan Laboratories, Indianapolis, IN, USA) and filtered tap water were provided *ad libitum* throughout the experiment. Procedures outlined in this study were approved by the Ohio State University Institutional Animal Care and Use Committee and adhered to the National Institutes of Health Guide for the Use and Care of Laboratory Animals.

Sleep deprivation

To deprive hamsters of sleep, the multiple platform procedure was used (Machado et al., 2004). Briefly, this method involves placing animals in polycarbonate cages that have six circular PVC caps (1 inch diameter) acting as platforms, which were affixed to the bottom of the cage using PVC cement. Platforms were spaced apart to prevent hamsters from sleeping across them. Tap water was added to the cage to partially submerge the platforms and warmed by a heating pad underneath the cage in case hamsters came into contact with water. Food and water were available *ad libitum* in these modified cages.

To assess the effect of sleep deprivation upon inflammatory responses, half of the LP and SP hamsters (N=20, both groups) were subjected to 24 h of sleep deprivation starting at 14:00 (lights off). The remaining hamsters were not deprived of sleep; they were briefly handled and then returned to their home cage. Use of home cage as the control condition (Weil et al., 2009; Zager et al., 2009) ensures that sleep deprivation is not occurring.

Lipopolysaccharide challenge

After 18 h (08:00 the next day), SP and LP hamsters that were sleep-deprived and non-sleep-deprived received an intraperitoneal injection of either bacterial LPS [25 μg or ca. 0.83 mg LPS kg^{-1} body mass (in a 35 g hamster); serotype 026:B6, Sigma-Aldrich, St Louis, MO, USA] dissolved in 0.9% saline, or 0.9% saline (vehicle). Hamsters were then returned to their respective cages (modified platform cage or home cage).

Tissue collection

At 6 h post-injection (14:00, lights off), hamsters were deeply anesthetized with isoflurane vapors and blood was collected from the retro-orbital sinus and placed on ice. This sample was obtained <3 min of initial handling. Hamsters were then killed by rapid decapitation, and liver, brain and reproductive tissues were collected. Body mass was not ascertained because some of the sleep-deprived hamsters had wet pelage, which skewed body mass measurements. Liver was immediately frozen on dry ice and stored at −80°C whereas brain was stored in RNAlater (Life Technologies, Grand Island, NY, USA) at 4°C. Blood was centrifuged for 30 min at 3000 ***g***, and plasma was drawn off with a pipette and stored at −80°C for later analysis. The hypothalamus was dissected from each brain and also stored at −80°C.

RNA extraction

Total RNA was extracted from hypothalami and liver using an RNeasy Micro Kit (#74106, Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. A Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to quantify the concentration of total RNA. The Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase Kit (Invitrogen, Carlsbad, CA, USA) was used to synthesize cDNA from 1 μg of total RNA. The cDNA was stored at −80°C before use.

Real-time PCR

Amplification was performed on an ABI 7500 Fast Real-Time PCR system using Taqman Universal PCR Master Mix (Life Technologies). Amplification conditions involved universal two-step real-time PCR cycling: 50°C for 2 min, 95°C for 10 min followed by 40 cycles of 95°C for 15 s, and 60°C for 1 min. We used IL-1 and TNF primers and probes that were previously reported from our laboratory (Pyter et al., 2005). Cytokine probes were labeled with the fluorescent reporter dye 6-FAM at the 5′ end and the

quencher MGB at the 3′ end: IL-1β forward 5′-CCAAGG-CCACAGGTATCTTGTT-3′, IL-1β reverse 5′-ACCTCAATGG-ACAGAATATAAACCAA-3′, IL-1β probe 5′-CCTGTACAAA-GCTCATG-3′, TNFα forward 5′- GAGCCAGCGTGCGAATG-3′, TNFα reverse 5′-AGCTGGTTGTCCTTGAGAGACAT-3′, TNFα probe 5′-CCTCCTGGCCAATGG-3′. Samples were run in duplicate and relative gene expression was calculated by comparison with a standard curve consisting of serial dilutions of pooled cDNA (1:1, 1:10, 1:100, 1:1000, 1:10,000) followed by normalization to 18s rRNA levels, which were measured using a Taqman primer and probe set (labeled with VIC; Life Technologies).

Radioimmunoassay

Cortisol is the main circulating glucocorticoid in Siberian hamsters (Reburn and Wynne-Edwards, 1999). Concentration in plasma was measured using a Cortisol ^{125}I radioimmunoassay kit (ImmuChem Coated Tube, MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's protocol. Intra-assay variation was 9.4% and the minimum detection limit was 1.19 ng ml^{-1}.

Statistical analysis

A three-way multi-factorial ANOVA was conducted to examine main effects of photoperiod (LP *versus* SP), sleep deprivation (deprived *versus* non-deprived), treatment (LPS) or vehicle [saline (SAL)] and their interactions upon reproductive organ mass, IL-1 and TNF gene expression in the liver and hypothalamus, and baseline cortisol concentration in blood plasma. Logarithmic transformation was necessary to satisfy assumptions of homogeneity of variances when analyzing IL-1 and TNF gene expression data. If significant effects or interactions were detected, then Tukey–Kramer *post hoc* tests were conducted to evaluate specific differences among groups. The α level was set at 0.05, and all values were expressed as means ± s.e.m.

RESULTS

Reproductive tissue mass

Reproductive tissue and epididymal fat pad masses were significantly greater in LP compared with SP hamsters (Table 1). Neither sleep deprivation nor endotoxin treatment significantly affected this difference (Table 1).

IL-1 gene expression

LPS treatment significantly increased IL-1 mRNA expression in both liver and hypothalamus compared with saline-injected controls (log-transformed data: liver, $P<0.05$; hypothalamus, $P<0.05$; Fig. 1A,C). There were significant interactions between treatment and sleep state (deprived or non-deprived) for IL-1 expression in the liver and hypothalamus ($P<0.05$). Specifically, sleep-deprived hamsters injected with LPS displayed reduced levels of IL-1 mRNA compared with non-sleep deprived hamsters injected with LPS; however, among saline-injected hamsters, IL-1 mRNA was not affected by sleep state (Tukey–Kramer tests, non-sleep-deprived + LPS > sleep deprived + LPS > non-sleep-deprived + SAL = sleep deprived + SAL, $P<0.05$; Fig. 1A,C). Other effects or interactions examined were not statistically significant.

TNF gene expression

LPS significantly elevated levels of TNF mRNA in both liver and hypothalamus compared with saline-treated controls (log-transformed data, $P<0.05$; Fig. 1B,D). There was a significant effect of photoperiod upon hypothalamic TNF gene expression ($P<0.05$), with SP hamsters, on average, exhibiting decreased levels of TNF mRNA compared with LP hamsters (Tukey–Kramer test, $P<0.05$). Other effects or interactions examined were not statistically significant.

Plasma cortisol

Plasma cortisol concentration was not elevated by 24 h of sleep deprivation (Fig. 2). Photoperiod significantly influenced baseline cortisol levels ($P<0.05$), and this difference was not dependent upon sleep state or injection type (Fig. 2). *Post hoc* analysis revealed that SP hamsters had higher baseline cortisol levels (129.75±14.49 ng ml^{-1}) than LP hamsters (76.91±10.67 ng ml^{-1}; Tukey–Kramer test, $P<0.05$). Other effects or interactions examined were not statistically significant.

DISCUSSION

This study provides molecular evidence that sleep deprivation suppresses IL-1-dependent pathways that trigger inflammation in the periphery and hypothalamus, an important area in the brain that regulates the acute phase response to infection (Blatteis, 1988; Guijarro et al., 2006).

Sleep deprivation suppresses peripheral and hypothalamic IL-1 gene expression following endotoxin treatment

These results are consistent with previous research in mice that has documented suppression of the inflammatory responses to an immunogen after 48 h of sleep deprivation using the same modified platform method (Weil et al., 2009). Although LPS treatment elevated TNF mRNA levels in the liver and hypothalamus compared with saline injection, sleep deprivation did not attenuate this increase, which implies that TNF-mediated inflammation is less

Table 1. Photoperiodic differences in reproductive tissue mass in male Siberian hamsters

		Reproductive tissue mass (g)			
Experimental group	*N*	Paired testes	Epididymides	Epididymal white adipose tissue	Seminal vesicles
LP + SAL + normal sleep	10	0.660±0.046^{a}	0.571±0.139^{a}	0.570±0.158^{a}	0.198±0.028^{a}
LP + SAL + sleep deprived	10	0.672±0.020^{a}	0.483±0.119^{a}	0.521±0.126^{a}	0.173±0.024^{a}
LP + LPS + normal sleep	10	0.717±0.052^{a}	0.531±0.127^{a}	0.544±0.135^{a}	0.220±0.028^{a}
LP + LPS + sleep deprived	10	0.669±0.026^{a}	0.445±0.103^{a}	0.473±0.124^{a}	0.162±0.018^{a}
SP + SAL + normal sleep	10	0.073±0.017^{b}	0.149±0.040^{b}	0.063±0.014^{b}	0.056±0.012^{b}
SP + SAL + sleep deprived	10	0.102±0.052^{b}	0.143±0.037^{b}	0.091±0.031^{b}	0.048±0.007^{b}
SP + LPS + normal sleep	10	0.093±0.017^{b}	0.155±0.054^{b}	0.074±0.016^{b}	0.057±0.009^{b}
SP + LPS + sleep deprived	10	0.098±0.018^{b}	0.168±0.039^{b}	0.103±0.033^{b}	0.061±0.010^{b}

Values are expressed as means ± s.e.m. The overall three-way ANOVA indicated a significant effect of photoperiod (all $P<0.05$) for each tissue type. Shared letters indicate no significant differences among groups using Tukey–Kramer *post hoc* tests.
Abbreviations: LP, long photoperiod; LPS, lipopolysaccharide; SAL, saline; SP, short photoperiod.

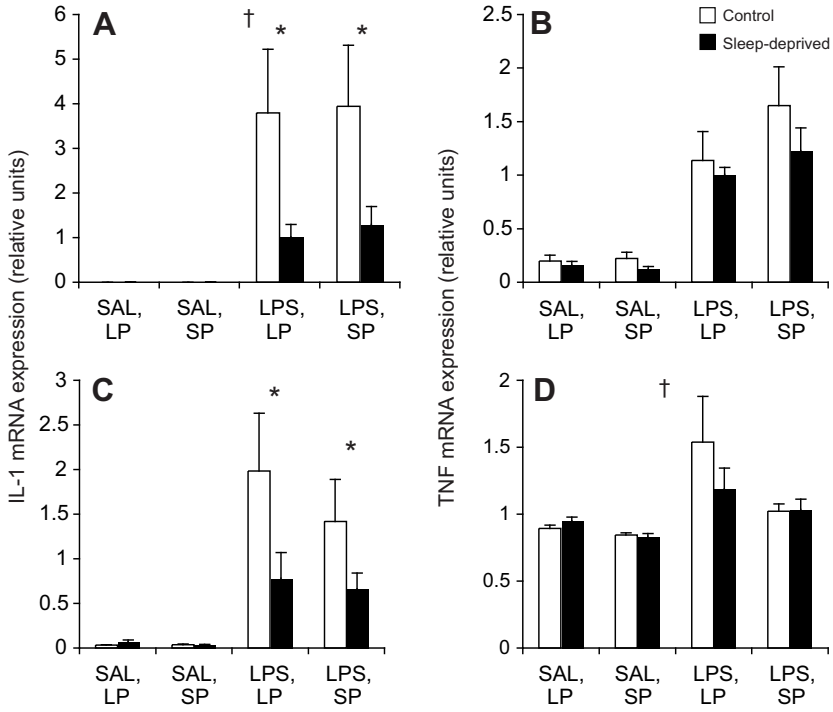

Fig. 1. Interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF) mRNA expression (means ± s.e.m.) in control (white) and sleep-deprived (black) Siberian hamsters adapted to long photoperiod (LP) or short photoperiod (SP) and then injected with saline (SAL) or lipopolysaccharide (LPS). IL-1 (A) and TNF (B) gene expression from liver, and IL-1 (C) and TNF (D) gene expression from hypothalamus. *N*=9 or 10 in each group. Asterisk denotes a significant difference (P<0.05) between LPS and SAL groups. Dagger indicates an overall effect of photoperiod (P<0.05).

sensitive to the immunosuppressive effects of sleep deprivation compared with IL-1. Alternatively, the temporal dynamics of suppression may have differed between TNF and IL-1, such that TNF suppression occurred, but at a different time point that was not assessed in this study. This possibility is not supported by a previous study in female Siberian hamsters that reported similar temporal profiles of IL-1 and TNF mRNA expression after LPS challenge, with maximum levels occurring 6 h after injection (Pyter et al., 2005). Among humans, sex differences in inflammatory responses after one night of sleep loss are apparent (Irwin et al., 2010). Whether such sex differences occur in Siberian hamsters and other rodents remains unspecified.

Although a reduction in IL-1 gene expression following sleep loss was not associated with glucocorticoids in this study, other components of the neuroendocrine response to stress, such as corticotropin-releasing hormone, adrenocorticotropic hormone or catecholamines, have immunoregulatory functions and could conceivably alter pro-inflammatory cytokine activation (Meerlo et al., 2002; Andersen et al., 2005). Additional candidates that deserve further study and are not directly involved with the stress response are growth hormone, leptin and ghrelin. These metabolic hormones regulate the immune system (Hattori, 2009; Baatar et al., 2011; Carlton et al., 2012) but are also altered by experimental sleep loss (Mullington et al., 2009).

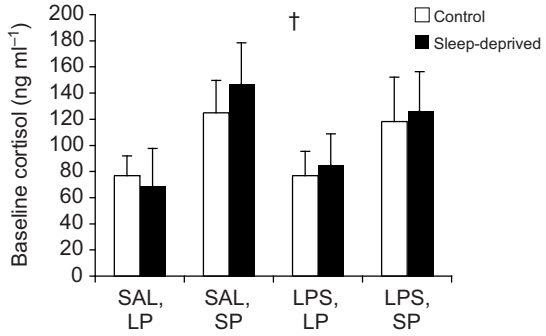

Fig. 2. Baseline cortisol levels (ng ml^{-1}) of control (white) and sleep-deprived (black) Siberian hamsters adapted to long photoperiod (LP) or short photoperiod (SP) and then injected with saline (SAL) or lipopolysaccharide (LPS). *N*=9 or 10 in each group. Dagger indicates an overall effect of photoperiod (P<0.05).

In the absence of real or simulated infection, acute and chronic sleep loss has been demonstrated to increase local and peripheral levels of pro-inflammatory cytokines, which is thought to contribute to the etiology of diseases associated with chronic low-grade inflammation (Simpson and Dinges, 2007; Mullington et al., 2009). However, in this study, 24 h of sleep deprivation did not increase cytokine expression among saline-injected hamsters. This result suggests that the immune systems of Siberian hamsters are less prone to the pro-inflammatory effects of acute sleep deprivation than other species. Additional studies that increase sleep duration and measure other inflammatory mediators associated with low-grade inflammation (plasma cytokines, C-reactive protein, etc.) in Siberian hamsters would be useful to better understand the temporal dynamics of the inflammatory response to sleep loss. Furthermore, suppression of IL-1 gene expression in the hypothalamus from sleep deprivation could be influenced by microglial activation in the central nervous system. Microglia have been shown to promote inflammation in the central nervous system (Liu and Hong, 2003). These cells assume an activated state when induced by LPS and rapidly produce pro-inflammatory cytokines, such as IL-1, IL-6 and TNF (Hanisch, 2002). The influence of sleep loss upon microglia-induced neuroinflammation is poorly understood (Wisor et al., 2011), and represents a promising area of research.

Lack of photoperiodic effects

The original hypothesis that exposure to short days attenuates suppression of LPS-induced inflammatory responses following sleep deprivation compared with long days was not supported. Photoperiod did not affect IL-1 or TNF mRNA expression in the

liver or hypothalamus in sleep-deprived hamsters, which implies that sleep deprivation is equally effective at suppressing IL-1-dependent inflammation under different photoperiods. This result cannot be attributed to variation in responsiveness to photoperiod because SP hamsters had significantly lower reproductive organ and fat pad masses than LP hamsters, which demonstrates that photoperiod manipulations were effective. In addition, hamsters that were reproductively non-responsive to SP were excluded from the experiment. Although SP hamsters display extended nocturnal activity periods and reduced EEG intensity compared with LP hamsters (Deboer and Tobler, 1996; Warner et al., 2010), duration of sleep is not correspondingly shortened (Deboer and Tobler, 1996; Deboer et al., 2000; Ashley et al., 2012). As is the case with most nocturnal rodents, Siberian hamsters exhibit a seasonal shift in timing, distribution and consolidation of sleep, but the total daily duration remains unchanged (Franken et al., 1995; Deboer and Tobler, 1996; Deboer et al., 2000; Ashley et al., 2012). This suggests that hamsters adapted to LP and SP are responding similarly to sleep deprivation because their durations of normal sleep in a 24 h period are equivalent and their homeostatic responses to acute sleep deprivation (4 h) are similar (Deboer and Tobler, 1996).

Among non-sleep-deprived hamsters, there was a significant effect of photoperiod upon hypothalamic TNF gene expression, with SP hamsters displaying lower levels than LP hamsters. It has been previously shown that short photoperiods attenuate hypothalamic IL-1 and TNF mRNA levels in female hamsters relative to long photoperiods (Pyter et al., 2005). As discussed, because our study used male hamsters, it is possible that a lack of photoperiodic differences in IL-1 pro-inflammatory gene expression can be attributed to sexual differences in temporal dynamics of the inflammatory response (Irwin et al., 2010). Testing this hypothesis in hamsters will require further study.

Photoperiodic differences in inflammatory responses appear to be partially regulated by the pineal hormone melatonin (Bilbo and Nelson, 2002). Because melatonin is produced and secreted at night, hamsters adapted to SP (and thus long nights) display increased nightly duration of melatonin secretion compared with hamsters adapted to LP (short nights). Furthermore, melatonin injections timed to mimic the short-day profile of melatonin secretion reduces LPS-induced fever in long-day-adapted hamsters, which suggests an inhibitory action of melatonin upon LPS-induced inflammation (Bilbo and Nelson, 2002). Because total sleep deprivation or chronic sleep restriction tends to blunt or interfere with melatonin rhythms (Zeitzer et al., 2007; Rogers and Dinges, 2008), it is conceivable that this dysregulation could have negated the anti-inflammatory effect of melatonin in SP hamsters, leading to a lack of photoperiodic differences in pro-inflammatory gene expression. An investigation into the effect of sleep deprivation upon daily melatonin profiles in relation to photoperiod is necessary to further assess this hypothesis.

Suppression of peripheral and hypothalamic inflammation independent of cortisol

Deprivation of sleep for 24 h using the modified multiple platform method did not increase baseline cortisol concentration compared with non-sleep-deprived hamsters despite an effect of sleep deprivation upon cytokine gene expression. This result is consistent with studies in rats that demonstrate elevation of baseline glucocorticoid concentrations after 48 h of sleep deprivation or 7 days of sleep restriction, but not after 24 h of sleep deprivation using the modified multiple platform method (Zager et al., 2007).

These results provide evidence that the suppressive effects of sleep loss upon LPS-induced inflammation are not mediated by glucocorticoids. Although several studies have implicated stress hormones as an important mediator of homeostatic and immunologic functions during dysregulated sleep (Meerlo et al., 2002; Andersen et al., 2005; Faraut et al., 2012), other studies have reported a lack of an association between immune function and circulating glucocorticoids following sleep loss (Born et al., 1997; Redwine et al., 2000; Bryant et al., 2004; Zielinski et al., 2012). In addition, blocking glucocorticoid receptors using mifepristone does not alter the suppressive effect of sleep deprivation upon LPS-induced pro-inflammatory gene or protein expression (Weil et al., 2009).

Photoperiod altered baseline cortisol levels irrespective of endotoxin treatment and regardless of whether hamsters were sleep deprived. Among vertebrates, LPS stimulates a rapid activation of the hypothalamic-pituitary-adrenal axis, which results in an elevation of glucocorticoid concentrations that reach maximum levels 1–2 h after injection, and then rapidly decline (Bilbo et al., 2003; Owen-Ashley et al., 2006). Because blood samples were collected 6 h after LPS injection, it is likely that this sampling time did not capture the LPS-induced rise in cortisol. SP hamsters had higher baseline cortisol than LP hamsters, and this photoperiodic difference in Siberian hamsters has been demonstrated in previous studies (Bilbo and Nelson, 2003; Pawlak et al., 2009), but not in others (Weil et al., 2007; Prendergast et al., 2009; Walton et al., 2012). Reasons for these discrepancies are unclear, but could be related to housing conditions or differences in experimental methodology, as baseline concentration should reflect energy balance.

Conclusions

Photoperiod had no effect upon the suppressive effect of sleep deprivation upon IL-1 gene expression in the periphery or hypothalamus, and sleep deprivation did not alter TNF mRNA levels among LPS-challenged hamsters. Furthermore, baseline cortisol was not elevated after 24 h of sleep deprivation, suggesting that this immunomodulatory hormone is not involved in mediating the suppression of the inflammatory response in sleep-deprived hamsters. Lastly, understanding the precise mechanisms that underlie altered inflammation during reduced or absent sleep will help elucidate new therapies to treat emerging diseases and syndromes associated with chronically sleep-deprived populations.

LIST OF ABBREVIATIONS

EEG	electroencephalographic
IL-1	interleukin-1
LP	long photoperiod
LPS	lipopolysaccharide
SAL	saline
SP	short photoperiod
TNF	tumor necrosis factor-alpha

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AUTHOR CONTRIBUTIONS

N.T.A., Z.M.W., U.J.M. and R.J.N. conceived and designed the study; N.T.A., J.C.W., A.H., N.Z., L.A.P., A.M.F. and R.A.L., carried out the study; N.T.A. analyzed the data; and N.T.A., J.C.W., Z.M.W., U.J.M. and R.J.N. wrote and edited the manuscript.

COMPETING INTERESTS

No competing interests declared.

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REFERENCES

Andersen, M. L., Martins, P. J., D'Almeida, V., Bignotto, M. and Tufik, S. (2005). Endocrinological and catecholaminergic alterations during sleep deprivation and recovery in male rats. *J. Sleep Res.* **14**, 83-90.

Ashley, N. T., Zhang, N., Weil, Z. M., Magalang, U. J. and Nelson, R. J. (2012). Photoperiod alters duration and intensity of non-rapid eye movement sleep following immune challenge in Siberian hamsters (*Phodopus sungorus*). *Chronobiol. Int.* **29**, 683-692.

Baatar, D., Patel, K. and Taub, D. D. (2011). The effects of ghrelin on inflammation and the immune system. *Mol. Cell. Endocrinol.* **340**, 44-58.

Beyaert, L., Greggers, U. and Menzel, R. (2012). Honeybees consolidate navigation memory during sleep. *J. Exp. Biol.* **215**, 3981-3988.

Bilbo, S. D. and Nelson, R. J. (2002). Melatonin regulates energy balance and attenuates fever in Siberian hamsters. *Endocrinology* **143**, 2527-2533.

Bilbo, S. D. and Nelson, R. J. (2003). Sex differences in photoperiodic and stress-induced enhancement of immune function in Siberian hamsters. *Brain Behav. Immun.* **17**, 462-472.

Bilbo, S. D., Drazen, D. L., Quan, N., He, L. and Nelson, R. J. (2002). Short day lengths attenuate the symptoms of infection in Siberian hamsters. *Proc. Biol. Sci.* **269**, 447-454.

Bilbo, S. D., Quan, N., Prendergast, B. J., Bowers, S. L. and Nelson, R. J. (2003). Photoperiod alters the time course of brain cyclooxygenase-2 expression in Siberian hamsters. *J. Neuroendocrinol.* **15**, 958-964.

Blatteis, C. M. (1988). Neural mechanisms in the pyrogenic and acute-phase responses to interleukin-1. *Int. J. Neurosci.* **38**, 223-232.

Born, J., Lange, T., Hansen, K., Mölle, M. and Fehm, H. L. (1997). Effects of sleep and circadian rhythm on human circulating immune cells. *J. Immunol.* **158**, 4454-4464.

Bryant, P. A., Trinder, J. and Curtis, N. (2004). Sick and tired: does sleep have a vital role in the immune system? *Nat. Rev. Immunol.* **4**, 457-467.

Carlton, E. D., Demas, G. E. and French, S. S. (2012). Leptin, a neuroendocrine mediator of immune responses, inflammation, and sickness behaviors. *Horm. Behav.* **62**, 272-279.

Deboer, T. and Tobler, I. (1996). Shortening of the photoperiod affects sleep distribution, EEG and cortical temperature in the Djungarian hamster. *J. Comp. Physiol. A* **179**, 483-492.

Deboer, T., Vyazovskiy, V. V. and Tobler, I. (2000). Long photoperiod restores the 24-h rhythm of sleep and EEG slow-wave activity in the Djungarian hamster (*Phodopus sungorus*). *J. Biol. Rhythms* **15**, 429-436.

Faraut, B., Boudjeltia, K. Z., Vanhamme, L. and Kerkhofs, M. (2012). Immune, inflammatory and cardiovascular consequences of sleep restriction and recovery. *Sleep Med. Rev.* **16**, 137-149.

Fonken, L. K., Bedrosian, T. A., Michaels, H. D., Weil, Z. M. and Nelson, R. J. (2012). Short photoperiods attenuate central responses to an inflammogen. *Brain Behav. Immun.* **26**, 617-622.

Franken, P., Tobler, I. and Borbély, A. A. (1995). Varying photoperiod in the laboratory rat: profound effect on 24-h sleep pattern but no effect on sleep homeostasis. *Am. J. Physiol.* **269**, R691-R701.

Frey, D. J., Fleshner, M. and Wright, K. P., Jr (2007). The effects of 40 hours of total sleep deprivation on inflammatory markers in healthy young adults. *Brain Behav. Immun.* **21**, 1050-1057.

Guijarro, A., Laviano, A. and Meguid, M. M. (2006). Hypothalamic integration of immune function and metabolism. *Prog. Brain Res.* **153**, 367-405.

Hanisch, U. K. (2002). Microglia as a source and target of cytokines. *Glia* **40**, 140-155.

Hattori, N. (2009). Expression, regulation and biological actions of growth hormone (GH) and ghrelin in the immune system. *Growth Horm. IGF Res.* **19**, 187-197.

Heldmaier, G. and Steinlechner, S. (1981). Seasonal control of energy requirements for thermoregulation in the djungarian hamster (*Phodopus sungorus*), living in natural photoperiod. *J. Comp. Physiol. B* **142**, 429-437.

Irwin, M., McClintick, J., Costlow, C., Fortner, M., White, J. and Gillin, J. C. (1996). Partial night sleep deprivation reduces natural killer and cellular immune responses in humans. *FASEB J.* **10**, 643-653.

Irwin, M. R., Wang, M., Ribeiro, D., Cho, H. J., Olmstead, R., Breen, E. C., Martinez-Maza, O. and Cole, S. (2008). Sleep loss activates cellular inflammatory signaling. *Biol. Psychiatry* **64**, 538-540.

Irwin, M. R., Carrillo, C. and Olmstead, R. (2010). Sleep loss activates cellular markers of inflammation: sex differences. *Brain Behav. Immun.* **24**, 54-57.

Jones, S. G., Paletz, E. M., Obermeyer, W. H., Hannan, C. T. and Benca, R. M. (2010). Seasonal influences on sleep and executive function in the migratory white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *BMC Neurosci.* **11**, 87.

Liu, B. and Hong, J. S. (2003). Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. *J. Pharmacol. Exp. Ther.* **304**, 1-7.

Machado, R. B., Hipólide, D. C., Benedito-Silva, A. A. and Tufik, S. (2004). Sleep deprivation induced by the modified multiple platform technique: quantification of sleep loss and recovery. *Brain Res.* **1004**, 45-51.

Martin, L. B., Weil, Z. M. and Nelson, R. J. (2008). Seasonal trade-offs between reproduction and immune activity. *Philos. Trans. R. Soc. B* **363**, 321-339.

Meerlo, P., Koehl, M., van der Borght, K. and Turek, F. W. (2002). Sleep restriction alters the hypothalamic-pituitary-adrenal response to stress. *J. Neuroendocrinol.* **14**, 397-402.

Mullington, J. M., Haack, M., Toth, M., Serrador, J. M. and Meier-Ewert, H. K. (2009). Cardiovascular, inflammatory, and metabolic consequences of sleep deprivation. *Prog. Cardiovasc. Dis.* **51**, 294-302.

Nelson, R. J. (1987). Photoperiod-nonresponsive morphs: a possible variable in microtine population density fluctuations. *Am. Nat.* **130**, 350-369.

Nelson, R. J. (2004). Seasonal immune function and sickness responses. *Trends Immunol.* **25**, 187-192.

Nelson, R. J. and Demas, G. E. (1996). Seasonal changes in immune function. *Q. Rev. Biol.* **71**, 511-548.

Nelson, R. J., Demas, G. E., Klein, S. L. and Kriegsfeld, L. J. (2002). *Seasonal Patterns of Stress, Immune Function and Disease*. Cambridge: Cambridge University Press.

Owen-Ashley, N. T., Turner, M., Hahn, T. P. and Wingfield, J. C. (2006). Hormonal, behavioral, and thermoregulatory responses to bacterial lipopolysaccharide in captive and free-living white-crowned sparrows (*Zonotrichia leucophrys gambelii*). *Horm. Behav.* **49**, 15-29.

Pawlak, J., Golab, M., Markowska, M., Majewski, P. and Skwarlo-Sonta, K. (2009). Photoperiod-related changes in hormonal and immune status of male Siberian hamsters, *Phodopus sungorus*. *Comp. Biochem. Physiol.* **152A**, 299-303.

Prendergast, B. J., Galang, J., Kay, L. M. and Pyter, L. M. (2009). Influence of the olfactory bulbs on blood leukocytes and behavioral responses to infection in Siberian hamsters. *Brain Res.* **1268**, 48-57.

Puchalski, W. and Lynch, G. R. (1986). Evidence for differences in the circadian organization of hamsters exposed to short day photoperiod. *J. Comp. Physiol. A* **159**, 7-11.

Pyter, L. M., Samuelsson, A. R., Quan, N. and Nelson, R. J. (2005). Photoperiod alters hypothalamic cytokine gene expression and sickness responses following immune challenge in female Siberian hamsters (*Phodopus sungorus*). *Neuroscience* **131**, 779-784.

Reburn, C. J. and Wynne-Edwards, K. E. (1999). Hormonal changes in males of a naturally biparental and a uniparental mammal. *Horm. Behav.* **35**, 163-176.

Redwine, L., Hauger, R. L., Gillin, J. C. and Irwin, M. (2000). Effects of sleep and sleep deprivation on interleukin-6, growth hormone, cortisol, and melatonin levels in humans. *J. Clin. Endocrinol. Metab.* **85**, 3597-3603.

Rogers, N. L. and Dinges, D. F. (2008). Interaction of chronic sleep restriction and circadian system in humans. *J. Sleep Res.* **17**, 406-411.

Simpson, N. and Dinges, D. F. (2007). Sleep and inflammation. *Nutr. Rev.* **65 Suppl.**, 244-252.

van Leeuwen, W. M., Lehto, M., Karisola, P., Lindholm, H., Luukkonen, R., Sallinen, M., Härmä, M., Porkka-Heiskanen, T. and Alenius, H. (2009). Sleep restriction increases the risk of developing cardiovascular diseases by augmenting proinflammatory responses through IL-17 and CRP. *PLoS ONE* **4**, e4589.

Walton, J. C., Grier, A. J., Weil, Z. M. and Nelson, R. J. (2012). Photoperiod and stress regulation of corticosteroid receptor, brain-derived neurotrophic factor, and glucose transporter GLUT3 mRNA in the hippocampus of male Siberian hamsters (*Phodopus sungorus*). *Neuroscience* **213**, 106-111.

Warner, A., Jethwa, P. H., Wyse, C. A., l'anson, H., Brameld, J. M. and Ebling, F. J. (2010). Effects of photoperiod on daily locomotor activity, energy expenditure, and feeding behavior in a seasonal mammal. *Am. J. Physiol.* **298**, R1409-R1416.

Wehr, T. A. (1991). The durations of human melatonin secretion and sleep respond to changes in daylength (photoperiod). *J. Clin. Endocrinol. Metab.* **73**, 1276-1280.

Weil, Z. M., Workman, J. L. and Nelson, R. J. (2007). Housing condition alters immunological and reproductive responses to day length in Siberian hamsters (*Phodopus sungorus*). *Horm. Behav.* **52**, 261-266.

Weil, Z. M., Norman, G. J., Karelina, K., Morris, J. S., Barker, J. M., Su, A. J., Walton, J. C., Bohinc, S., Nelson, R. J. and DeVries, A. C. (2009). Sleep deprivation attenuates inflammatory responses and ischemic cell death. *Exp. Neurol.* **218**, 129-136.

Wisor, J. P., Schmidt, M. A. and Clegern, W. C. (2011). Evidence for neuroinflammatory and microglial changes in the cerebral response to sleep loss. *Sleep* **34**, 261-272.

Zager, A., Andersen, M. L., Ruiz, F. S., Antunes, I. B. and Tufik, S. (2007). Effects of acute and chronic sleep loss on immune modulation of rats. *Am. J. Physiol.* **293**, R504-R509.

Zager, A., Andersen, M. L., Lima, M. M., Reksidler, A. B., Machado, R. B. and Tufik, S. (2009). Modulation of sickness behavior by sleep: the role of neurochemical and neuroinflammatory pathways in mice. *Eur. Neuropsychopharmacol.* **19**, 589-602.

Zager, A., Ruiz, F. S., Tufik, S. and Andersen, M. L. (2012). Immune outcomes of paradoxical sleep deprivation on cellular distribution in naive and lipopolysaccharide-stimulated mice. *Neuroimmunomodulation* **19**, 79-87.

Zeitzer, J. M., Duffy, J. F., Lockley, S. W., Dijk, D. J. and Czeisler, C. A. (2007). Plasma melatonin rhythms in young and older humans during sleep, sleep deprivation, and wake. *Sleep* **30**, 1437-1443.

Zielinski, M. R., Davis, J. M., Fadel, J. R. and Youngstedt, S. D. (2012). Influence of chronic moderate sleep restriction and exercise on inflammation and carcinogenesis in mice. *Brain Behav. Immun.* **26**, 672-679.