Published in final edited form as:

Physiol Behav. 2007 April 23; 90(5): 717–725. doi:10.1016/j.physbeh.2006.12.011.

Photoperiodic Regulation of Behavioral Responsiveness to Proinflammatory Cytokines

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Abstract

Symptoms of bacterial infection include decreases in body mass (cachexia), induction of depressive-like hedonic tone (anhedonia), decreases in food intake (anorexia), and increases in body temperature (fever). Recognition of bacteria by the innate immune system triggers the release of proinflammatory cytokines which induce these sickness behaviors via central and peripheral substrates. In Siberian hamsters, exposure to short day lengths decreases both the production of proinflammatory cytokines and the magnitude of the symptoms of infection. Short-day attenuation of sickness behaviors may arise solely from decreased production of cytokines; alternatively, substrates responsible for the generation of sickness behaviors may be less responsive to cytokines in short days. To discriminate among these hypotheses, Siberian hamsters were treated with either bacterial lipopolysaccharide (LPS; 25 μ g) or recombinant mouse IL-1 β (rIL-1 β ; 100 ng) following 11 weeks of exposure to long (15 h light/day) and short (9 h light/day) photoperiods. Replicating earlier work, the magnitude and/or duration of LPS-induced anorexia, anhedonia, cachexia, and fever were greater in long-day relative to short-day hamsters. A comparable short-day attenuation of sickness behaviors and fever was obtained in response to rIL-1 β treatment, despite treatment with identical concentrations of cytokine. These data suggest that hamsters subjected to immunoenhancing short days exhibit diminished symptoms of infection not solely because infections elicit lower levels of cytokine production, but also because the substrates upon which cytokines act become relatively refractory.

Keywords

seasonality; sickness behaviors; depression; anhedonia; food intake; body mass; fever; LPS; IL- β

Introduction

Annual variations in biotic (food availability) and abiotic (temperature) environmental factors impose constraints on reproduction and survival. Many species have evolved the ability to undergo seasonal changes in physiology and behavior (including, but not limited to, changes in reproductive behavior, energy balance, and immune function) in anticipation of such seasonal fluctuations [1–3]. In mammals, changes in day length (photoperiod) are sufficient to initiate or synchronize many of these physiological transitions [4].

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Siberian hamsters (*Phodopus sungorus*), in common with other nontropical rodents, exhibit reproductive involution following many weeks of exposure to naturally-decreasing or short photoperiods (< 13 h light/day; 4). Alongside changes in reproductive physiology, hamsters exposed to short photoperiods exhibit diverse changes in the immune system, including increases in leukocyte counts [5] and decreases in antibody and proinflammatory cytokine production [6–9], to list but a few.

Although one major function of the immune system is to keep pathogens from colonizing the host, bacterial infections often occur. Infected (sick) animals exhibit fever and profound changes in behavior and physiology shortly after the recognition of bacteria by the innate immune system [10,11]. These “sickness behaviors” are ubiquitous in vertebrates, and include reductions in ingestive, social, and sexual behaviors, induction of an anhedonic state, lethargy, and fever [11]. These transient changes in behavior—and changes in the neural and physiological substrates that control these behaviors—forestall the replication of bacteria during the hours immediately following infection, facilitate leukocytogenesis, and are critical to survival [11,12]. Sickness behaviors in response to gram-negative bacterial infections are initiated by the host’s immune system. Following recognition of the bacterial lipopolysaccharide (LPS) outer coat, macrophages produce pro- (IL-1 β , IL-6, and TNF- α) and anti- (IL-10, IL-1ra) inflammatory cytokines, which act on neural and peripheral targets to induce changes in physiology and motivational state [13–15]. Each of these proinflammatory cytokines induces specific sickness behaviors (e.g., peripheral injections of IL-1 diminish exploratory and social motivation [16,17], and induce anorexia [18] and fever [13]).

In hamsters, winter photoperiods robustly attenuate sickness behaviors; this curtailment of energetically-costly behavior has been argued to be an adaptive response to winter in small-bodied mammals [3]. Following a simulated infection (treatment with LPS), hamsters housed in short days exhibit shorter fevers relative to those housed in long days [7]; the magnitude and duration of anorexia, cachexia, and anhedonia are likewise attenuated in short days [19–20]. Accompanying the abrogation of sickness behaviors are decreases in peripheral production of IL-1 β , IL-6, and TNF- α [7,21] and in brain production of IL-1 β and TNF- α [22]. Mechanistically, one possible interpretation of these data is that the entire inhibitory effect of short photoperiod on the magnitude of sickness behaviors is simply a result of the short-day decrease in LPS-induced cytokine production. If this is true, then the level of cytokine produced by short-day leukocytes may simply be inadequate to cause high and sustained levels of anorexia, anhedonia, and fever. However, in light of the numerous reports that changes in day length simultaneously alter both production of, and behavioral responsiveness to, humoral signaling molecules (i.e., hormones [23–32]; reviewed in [2,33]), an alternative hypothesis emerges: seasonal changes in sickness behaviors may arise as a result of photoperiodic regulation of sensitivity to cytokines. If this alternative hypothesis is true, then photoperiodic differences in LPS-stimulated cytokine production may be of limited functional significance to the short-day amelioration of sickness behaviors; rather, substrates that mediate the effects of cytokines on behavior may be relatively refractory to cytokines in short days.

The purpose of this experiment was to evaluate a novel mechanism that may participate in the photoperiodic regulation of sickness behaviors, specifically, that changes in photoperiod alter behavioral and thermoregulatory responsiveness to cytokines. Photoperiodic differences in endogenous cytokine production were circumvented by providing hamsters housed in long- or short-day photoperiods with identical amounts of exogenous IL-1 β . Additional groups of hamsters were administered LPS to permit direct comparisons between the behavioral effects of LPS and those of a single proinflammatory cytokine.

Methods

Animals

Male Siberian hamsters (*Phodopus sungorus*) were from a breeding colony maintained at The University of Chicago. Hamsters were housed in polypropylene cages from birth in a room illuminated for 15 h per day with fluorescent light (15L, lights off 1700 h, Central Standard Time). Food (Harlan 8604 Teklad Rodent Diet) and filtered tap water were provided *ad libitum*, and temperature and humidity were held constant. Hamsters were weaned at 18–21 days of age and housed 1–4 animals per cage until experimental treatments began on week 0. All experimental procedures obtained prior approval by the Institutional Animal Care and Use Committee of The University of Chicago.

Photoperiod manipulations

On week 0, adult hamsters (between 2–3 months of age; $n=71$) were housed individually. Hamsters were then either transferred into a room illuminated for 9 h per day ($n=32$; short days, SD) or remained in their natal long day photoperiod ($n=39$; LD) for the next 15 weeks. The onset of darkness remained fixed at 1700 CST for both photoperiods.

Somatic and reproductive measures

Hamsters were weighed (to 0.1 g) on weeks 0, 9 and 15. Reproductive condition was assessed on weeks 0, 9, and 15 in lightly anesthetized hamsters by measuring the width and length of the left testis with analog calipers. The product of testis (length) $\times$ (width)² provided an estimated testis volume (ETV) which is highly correlated with testis weight and function in rodents [34,35]. On week 15, stage in the seasonal pelage color cycle was assessed in each hamster using an integer scale of 1 to 4 (1= dark “summer” fur, 4= white “winter” fur) [36]. In most laboratory populations of Siberian hamsters, there exist individuals that do not respond to short photoperiods with gonadal regression and SD-typical fur molt [37]. Because such photoperiod “nonresponders” sustain long-day-like reproductive and immune function in short photoperiods, we sought to exclude these individuals from all analyses. All animals from the SD photoperiod which sustained a decrease in ETV of >60% on weeks 9 and 15 were regarded as responsive to SD [7,34].

Surgical procedures

Calibrated temperature-sensitive data loggers (Thermochron iButton model DS1921H-F50; Maxim/Dallas Semiconductor, Dallas, TX) for recording body temperature (T_b) were implanted intra-abdominally under sodium pentobarbital-induced deep surgical anesthesia. Data loggers (“iButtons”) were coated with Elvax paraffin wax (Minimitter, Sunriver, OR), calibrated in a water bath over a physiologic range of temperatures, and sterilized prior to implantation. Calibration constants for each iButton were applied to all data collected. The temperature resolution of this model iButton is 0.125 °C, and it is sensitive over a range of temperatures from 15°C to 45°C. Each iButton was programmed to collect a T_b recording at 15 minute intervals for 20 successive days, beginning 2 weeks after surgical implantation. T_b data were stored on the iButton itself and were downloaded after harvesting the iButton at autopsy on week 15. Temperature data collected using this model iButton are strongly positively correlated with actual temperature ($R^2>0.99$), and these devices yield T_b records highly similar to those generated by widely used radiotelemetry devices (Minimitter ER-4000; [38]).

Drugs and preparation

E. coli lipopolysaccharide (LPS, from *E. coli* 026:B6; lypohyllized powder; Sigma; St. Louis, MO) was reconstituted in sterile 0.9% saline and diluted to a final concentration of 0.25 mg/

ml. Recombinant mouse IL-1 β (rIL-1 β ; R&D Systems; Minneapolis, MN) was reconstituted in sterile filtered 1X PBS with 0.1% BSA (Sigma) and diluted to a final concentration of 1 μ g/ml. LPS and rIL-1 β solutions were prepared fresh on the day of delivery.

Injections

On week 11 (*day 0*), 30 minutes before lights-off (at 1630 h CST), LD (n=38) and SD (n=21) hamsters were injected with either LPS (25 μ g in 0.1 ml sterile saline; LD: n=13, SD: n=7), rIL-1 β (100 ng in 0.1 ml PBS/BSA; LD: n=13, SD: n=8), or 0.1 ml PBS/BSA (saline; LD: n=12, SD: n=6). This amount of LPS is comparable to that used previously in this species [7, 39]. The amount of rIL-1 β used in this study was selected based on the results of a pilot study (data not shown) in which colonic temperature (T_c) of adult male hamsters was measured at 2–3 h intervals following i.p. injection of rIL-1 β (100 ng or 200 ng), LPS (25 μ g), or saline vehicle (n=5 hamsters per group). Relative to saline treatments, 100 ng rIL-1 β and 200 ng rIL-1 β each elicited significant 0.5 to 1.0°C increases in T_c , as did 25 μ g LPS (repeated-measures ANOVA: $p \leq 0.05$, all comparisons). However, T_c increases elicited by 100 ng rIL-1 β were statistically indistinguishable from those elicited by LPS treatment ($p > 0.40$), whereas the increase in T_c following 200 ng rIL-1 β treatment significantly exceeded that elicited by LPS ($p < 0.05$). Thus the lower of the two rIL-1 β doses (100 ng) which still mimicked the effects of LPS was selected for use in the following experiments.

Somatic and behavioral measurements

Three standard measures of sickness responses—cachexia (changes in body mass), anorexia (changes in food intake), and anhedonia (changes in consumption of sweetened condensed milk)—were assessed during the 48 hour interval following injection treatments.

Body mass responses were determined by weighing hamsters (± 0.1 g) immediately prior to injection (1630 h), and again at the same time on day +1 and day +2.

Beginning 5 days prior to injections (i.e., day –5), the contents of each hamster's food hopper was weighed (± 0.1 g) between 1600 h and 1700 h. Each cage was searched for fallen or cached food pellets, and hamsters were assessed for cheek pouching of food. The weight of the unconsumed food was recorded, and daily food intake was calculated as the change in the weight of the unconsumed food over the preceding 24 h interval. Food intake measurements continued daily through day +2.

Also beginning on day –5, hamsters were presented with a sweetened condensed milk solution (Borden brand; mixed 1:1 with filtered tap water) for the first six hours of the dark phase (1700 h to 2300 h). The ingestion of a highly-palatable sucrose or sweetened-condensed milk solutions is a standard assay for changes in hedonic tone; in animal models, decreases in ingestion are considered indicative of anhedonia [40]. Milk was presented in their home cage in a bottle that was positioned alongside their standard water bottle. Milk bottles were weighed (± 0.1 g) prior to presentation (at 1700 h), and again upon removal (at 2300 h). Six-hour milk intake was calculated as the change in weight of the bottles over the 6 h presentation interval. Milk presentation continued in the same manner daily through day +2.

Statistical analyses

Body mass and ETV values obtained on weeks 0 and 9 were compared using ANOVA. Because photoperiod significantly affected body mass and ingestive behavior prior to drug administration, changes in body mass, food intake, and milk intake following injections were each expressed as a percentage of individual baseline values (mean of the three daily measurements immediately preceding the injection) and were compared between groups using 2 (LD, SD) $\times$ 3 (LPS, rIL-1 β , saline) factorial ANOVA. Data collected between day 0 and day

+1 are referred to as “day +1” values, those collected between day +1 and day +2 are referred to as “day +2” values. Where permitted by a significant omnibus F value, pairwise comparisons were conducted using planned t-tests.

T_b values collected at 15 min intervals for 24 h following injections were smoothed using a 3-point moving average. Baseline (mean T_b during the 90 minutes preceding injection) differed significantly between photoperiod groups (see Results), thus direct comparisons of T_b responses to drug treatments between photoperiods were not warranted. Changes in T_b were therefore compared separately within each photoperiod using repeated measures ANOVA. Pairwise comparisons between injection groups at individual time points were then conducted using t-tests.

LPS and IL-1 β treatments result in sustained (>4–6 h) febrile responses. Idiographic analyses permitted specification of the latency to initiate, and duration of, febrile responses in individual animals as follows: Threshold values for identifying elevated T_b s in rIL-1 β - and LPS-injected hamsters were established based on the mean and variance in T_b s of saline-injected hamsters. T_b was considered elevated when it exceeded the 95% confidence limits of the mean T_b value of saline-injected hamsters at any given point in time. To avoid labeling transient fluctuations in T_b as meaningful elevations, only T_b values which together comprised a sustained increase in T_b (defined as >6 successive readings above the 95% confidence limit) were recorded as meaningful elevations in T_b . For each animal, the beginning of the first sustained increase in T_b following injection marked the onset of the febrile response, and the total amount of elevated T_b during the 24 h following injection defined the duration of the febrile response. The duration of fever was compared between groups using student's t-tests.

All statistical calculations were conducted using Statview 5.0. Differences were considered significant if $p \leq 0.05$.

Results

Somatic, reproductive, and behavioral responsiveness to photoperiod

Hamsters that either (1) failed to exhibit as fur molt of stage 2 or greater, or (2) failed to exhibit a decrease in ETV of >60% on weeks 9 and 15 were regarded as photoperiod nonresponders [34]. These criteria resulted in identification of 11 nonresponders, a proportion (34%) comparable to that reported for this species previously [41]; these animals were not used further in the study.

Hamsters exposed to SD had significantly lower body masses and testis sizes than LD hamsters after 9 weeks of photoperiod treatments (Table 1). Baseline values for daily food intake and 6 h consumption of sweetened condensed milk were significantly higher in LD relative to SD hamsters (Table 1). Within each photoperiod group, hamsters in the three different injection groups had comparable body masses, ETVs, baseline food intake, and baseline milk intake values prior to injection ($p > 0.05$, all comparisons).

LPS treatments

Anorexia—LD hamsters treated with LPS exhibited decreases in food intake relative to those injected with saline on day +1 ($t_{23}=6.00$, $p < 0.05$) and day +2 ($t_{23}=2.08$, $p < 0.05$). LPS did not significantly affect food intake in SD hamsters at any time after injection (day +1: $t_{11}=1.44$, $p > 0.05$; day +2: $t_{11}=0.61$, $p > 0.05$; Fig 1A). LPS-induced changes in food intake were significantly greater in LD relative to SD hamsters on day +1 ($t_{18}=5.51$, $p < 0.05$) and day +2 ($t_{18}=3.42$, $p < 0.05$; Fig. 1A).

Weight loss—LD hamsters injected with LPS exhibited significant decreases in body mass relative to saline-treated animals which were sustained through day +2 (day +1: $t_{23}=5.22$, $p<0.05$; day +2: day +1: $t_{23}=8.59$, $p<0.05$; Fig. 1B). LPS-treated SD hamsters exhibited significant decreases in body mass on day +1 ($t_{11}=3.29$, $p<0.05$), but body masses of these animals were comparable to those of saline-treated hamsters on day +2 ($t_{11}=1.4$, $p>0.05$). The effects of LPS on weight loss were comparable in magnitude among LD and SD hamsters on day +1 ($t_{18}=0.65$, $p>0.05$), but not on day +2 ($t_{18}=2.82$, $p<0.05$; Fig. 1B).

Anhedonia—Among LD hamsters, intake of sweetened condensed milk was significantly suppressed on day +1 ($t_{22}=13.9$, $p<0.05$) and day +2 ($t_{23}=6.35$, $p<0.05$) in LPS-treated relative to saline-treated animals; in SD hamsters, LPS treatment suppressed milk intake on day +1 ($t_{11}=5.17$, $p<0.05$), but this effect was not evident on day +2 ($t_{11}=0.48$, $p>0.05$; Fig. 1C). The magnitude of suppression of milk intake by LPS was comparable among LD and SD hamsters on day +1 ($t_{17}=0.48$, $p>0.05$), but was significantly greater in LD hamsters on day +2 ($t_{18}=4.42$, $p<0.05$; Fig. 1C).

Fever—In LD and SD hamsters, LPS treatments significantly altered the pattern of T_{bs} during the 24 h interval following injection (LD: $F_{87,2001}=4.76$, $p<0.05$; SD: $F_{87,957}=1.54$, $p<0.05$; Fig. 2A, 2B). The number of 15 min intervals during which T_{bs} of LPS treated hamsters significantly exceeded those of saline-treated hamsters differed between the two photoperiods. Among LD hamsters, LPS resulted in significantly elevated T_{bs} during 27 of the 15 min measurement intervals ($p<0.05$, all comparisons). Four of these measurements occurred during the dark phase immediately following LPS treatment (between 1930 h and 2030 h, inclusive); the majority ($n=23$) occurred surrounding the transition from the dark to the light phase (between 0215 h and 0815 h). In SD, T_{bs} of LPS-injected hamsters exceeded those of saline-treated hamsters during 14 measurement intervals ($p<0.05$, all comparisons). Half of these occurred during the dark phase following injection, and half were observed during the transition from dark to light. The duration of LPS-induced fever was significantly greater in LD (9.3 ± 1.9 h) relative to SD (3.2 ± 1.0 h) hamsters ($t_{18}=2.32$, $p<0.05$).

rIL-1 β treatments

Anorexia—LD hamsters treated with rIL-1 β exhibited decreases in food intake on day 1 relative to hamsters treated with saline ($t_{23}=2.37$, $p<0.05$); by day +2, food intake in LD-rIL-1 β -treated hamsters no longer differed from that of controls ($t_{23}=0.27$, $p>0.05$; Fig. 3A). rIL-1 β was without effect on food intake in SD hamsters on either day following injection (day +1: $t_{12}=1.34$, $p>0.05$; day +2: $t_{12}=1.31$, $p>0.05$). rIL-1 β suppressed food intake in LD relative to SD hamsters on day +1 ($t_{19}=6.38$, $p<0.05$) and day +2 ($t_{19}=4.21$, $p<0.05$; Fig. 3A).

Weight loss—Compared to controls, LD hamsters treated with rIL-1 β exhibited significant decreases in body mass which were sustained through day +2 (day +1: $t_{23}=4.59$, $p<0.05$; day +2: $t_{23}=4.78$, $p<0.05$; Fig. 3B). SD hamsters did not exhibit significant changes in body mass following rIL-1 β treatment (day +1: $t_{12}=0.11$, $p>0.05$; day +2: $t_{12}=1.10$, $p>0.05$). Changes in body mass following rIL-1 β treatment were significantly greater in LD relative to SD hamsters on day +1 ($t_{19}=2.95$, $p<0.05$) but not on day +2 ($t_{19}=0.77$, $p>0.05$; Fig. 3B).

Anhedonia—rIL-1 β significantly suppressed milk intake in LD hamsters on day +1 ($t_{23}=10.8$, $p<0.05$) and on day +2 ($t_{23}=3.42$, $p<0.05$); rIL-1 β treatments suppressed milk intake in SD hamsters on day +1 ($t_{12}=2.70$, $p<0.05$), but not on day +2 ($t_{12}=1.79$, $p>0.05$; Fig. 3C). Following rIL-1 β treatment, the magnitude suppression of milk intake was greater in LD relative to SD hamsters on day +1 ($t_{19}=2.58$, $p<0.05$) but not on day +2 ($t_{19}=0.32$, $p>0.05$; Fig. 3C).

Fever—rIL-1 β treatment affected the pattern of T_b change during the 24 h interval following injection (LD: $F_{87,2001}=2.98$, $p<0.05$; SD: $F_{87,1044}=1.80$, $p<0.05$; Fig. 4A, 4B). Among LD hamsters, rIL-1 β treatment resulted in significantly elevated T_b s during 21 measurement intervals ($p<0.05$, all comparisons). The majority ($n=13$) of these occurred during the dark phase immediately following injection (between 1915 h and 2215 h); the remainder occurred throughout the early portion of the following light phase. Among SD hamsters, T_b s of rIL-1 β -injected hamsters exceeded those of saline-treated hamsters during 10 measurement intervals ($p<0.05$, all comparisons), all of which occurred during the dark phase following injection. Fever duration exhibited by LD rIL-1 β -treated hamsters (5.94 ± 1.2 h) was significantly greater than that of SD (2.78 ± 0.7 h) hamsters ($t_{19}=1.87$, $p<0.05$).

Discussion

Recent work has established that LPS-induced sickness behaviors are suppressed in hamsters exposed to short days [7,17,19,39,42]. Coincident with this abrogation of sickness behaviors are reductions in the ability of LPS to elicit production of proinflammatory cytokines (IL-1 β , IL-6, and TNF- α [7,18,19]) in short-days. This short-day attenuation of sickness behaviors could reflect at least two separate processes: (1) the amount of cytokine produced in the periphery under short days may be below some putative threshold value necessary for engaging and sustaining maximal sickness behaviors, or (2) the thresholds of the substrates upon which cytokines act to generate sickness behaviors may have been raised in short days. In the present study, adaptation to short days significantly attenuated rIL-1 β -induced sickness behaviors. Exogenous IL-1 β —in amounts that mimicked the effects of LPS on anorexia, anhedonia, and fever during the first 24 h after injection—elicited more robust and enduring sickness behaviors in LD relative to SD hamsters. rIL-1 β inhibited food intake in LD hamsters but was without effect in SD hamsters. rIL-1 β -induced cachexia was smaller and less protracted among SD hamsters. Anhedonia was evident in both LD and SD hamsters following rIL-1 β treatment, but the magnitude and duration of the anhedonic state were significantly greater in LD relative to SD hamsters. Finally, the febrile response to rIL-1 β was over twice as long in LD hamsters. These outcomes indicate that physiological and behavioral responsiveness to cytokines are attenuated in hamsters exposed to SD. Although LPS treatments elicit smaller cytokine responses in SD relative to LD hamsters [7,18,19], such decreases in cytokine production may not be necessary for photoperiodic differences in the symptoms of infection to manifest. The data are consistent with the hypothesis that substrates responsible for the generation of sickness behaviors and fever are relatively refractory to cytokines following adaptation to SD.

A number of physiological mechanisms may participate in this change in cytokine sensitivity. These may involve changes in responsiveness to cytokines occurring centrally and/or in the periphery. Proinflammatory mediators such as LPS and IL-1 β induce fever and sickness behaviors through the downstream induction of additional cytokines (IL-1 β , TNF- α , and IL-6), both in the periphery and in the brain [43]. In addition, LPS and IL-1 β gain access to the CNS through multiple routes. A neural route involves recognition of peripheral proinflammatory stimuli by the vagus nerve [44–49], and a humoral route involves production of cytokines at circumventricular sites, diffusion across the blood-brain barrier, and activation of neural targets [15]. The present data may reflect photoperiodic changes in responsiveness to IL-1 β at any of the several stages between the initial generation of IL-1 β in the periphery and the ultimate recognition of proinflammatory mediators by neural targets that regulate behavior. This experiment did not aim to determine *where* changes in responsiveness to cytokines are occurring, but simply *whether* substrates differ in their responsiveness to IL-1 β following changes in day length. Further work, involving administration of proinflammatory cytokines exclusively to central substrates (e.g., via i.c.v. infusions) will likely permit discrimination among the above possibilities and specification of the relevant physiological sites.

Consistent with earlier work [7,17,18,39], ingestive, somatic, and hedonic symptoms of infection were more severe in LD relative to SD hamsters. Among treatment groups that exhibited significant sickness behaviors, treatment with LPS elicited either greater (anorexia, cachexia, anhedonia) or more enduring (anorexia) symptoms of infection relative to treatment with rIL-1 β . At least two explanations for this outcome are possible. First, the bolus injection of rIL-1 β used in this experiment is unlikely to yield a circulating IL-1 β profile that matches endogenous cytokine production following LPS treatment [46,50]. Secondly, multiple proinflammatory cytokines, including TNF- α [51] and IL-6 [52], contribute to the generation of sickness behaviors, both individually and synergistically [45]. It remains unclear whether photoperiod likewise modulates responsiveness to these cytokines.

Also consistent with earlier work [7,39], febrile responses to LPS and IL-1 β were less robust in SD relative to LD housed hamsters. Constrained febrile responses to LPS in SD hamsters have been associated with diminished production of COX-2 enzyme in the anterior hypothalamus, which limits prostaglandin production [42]. Peripheral administration of proinflammatory cytokines (IL-1 β , TNF- α) induces COX-2 production in forebrain microvasculature, circumventricular organs, and parenchymal cells of the hypothalamic paraventricular nucleus [53], suggesting that some or all of these sites may be relevant to the generation of sickness responses to LPS. In addition to the decreases in production of pyrogenic proinflammatory cytokines that occur in short days [7,18], the present work suggests that adaptation to SD attenuates responsiveness to one of these cytokines (i.e., IL-1 β). Thus, in common with behavioral responses to simulated infection, the thresholds of the substrates upon which cytokines act to induce and sustain febrile responses may be raised in SD.

These data suggest that male hamsters subjected to immunoenhancing short days exhibit diminished sickness behaviors, not solely because infections elicit lower levels of cytokine production, but also because the substrates upon which cytokines act are less responsive. Diminished responsiveness to rIL-1 β in SD hamsters is reminiscent of the decline in behavioral sensitivity of male Syrian hamsters to androgens after adaptation to SD [23,54]. In Syrian hamsters, castration eliminates detectable circulating testosterone and terminates sexual behavior in the majority of individuals housed in LD [55]. Likewise, several weeks of exposure to SD yields gonadal atrophy, substantial decreases in testosterone secretion, and marked reductions in copulatory behavior [23,54]. However, testosterone replacement therapy is significantly more effective in restoring copulatory behavior in castrates housed under LD as opposed to SD [23,56]. Analogous decrements in behavioral responsiveness (lordosis) of females to estradiol and progesterone following adaptation to SD have also been reported [24–26,29–32]. Thus in the reproductive neuroendocrine system, seasonal decreases in hormone production are accompanied by a parallel decrease in hormone responsiveness (i.e., raising of the threshold for activation of sex behavior by steroids in SD). Interactions between these two processes ultimately determine levels of sexual activity. The present experiments suggest that analogous phenomena may govern seasonal regulation of sickness behaviors: photoperiod dictates both the ability of LPS to elicit production of proinflammatory cytokines and behavioral responsiveness to these molecules.

The physiological relevance of systems governed jointly by (1) photoperiodic changes in the production of humoral mediators and (2) photoperiodic changes in sensitivity to these mediators is evident in the demonstration that transfer of SD-photoinhibited hamsters to intermediate-duration photoperiods is sufficient to induce testicular growth and restore gonadal androgen secretion, but unlike LD-housed animals with comparable testosterone concentrations, virtually all of these animals fail to complete an ejaculatory sequence [56]. In this paradigm, the intermediate-duration photoperiod is evidently regarded as a long day by systems that regulate the production of gonadal steroids, but as a short day by those that govern behavioral responsiveness to these hormones. In other words, the critical day lengths for these

processes differ. In the immune system, critical day lengths for induction of a SD phenotype have likewise been shown to vary on a trait-by-trait basis [57]. If seasonal transitions in cytokine production and in cytokine responsiveness are triggered by dissimilar critical day lengths, then the interaction between these two processes may figure prominently in regulating seasonal changes in the symptoms of infection.

Acknowledgments

The authors thank Tanita Mason, Randy Renstrom, Scott Baillie, Jerome Galang, and Curtis Wilkerson for expert technical assistance. This work was supported by grant AI-67406 from the National Institute of Allergy and Infectious Diseases.

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Figure 1.

Mean ($\pm$ SEM) changes in food intake (panel A), body weight (panel B), and sweetened condensed milk intake (expressed as a percentage of baseline values) of adult male Siberian hamsters housed in long (LD) or short day lengths (SD) during the two days (day +1, day +2) immediately following i.p. injection with 25 μ g bacterial lipopolysaccharide (LPS). * $p \leq 0.05$ vs. saline-injected group in the same photoperiod; # $p \leq 0.05$ vs. LPS-treated LD group.

Figure 2.

Mean ($\pm$ SEM) core body temperature (T_b) of adult male Siberian hamsters during the 3 hours prior to, and 24 hours after, i.p. treatment with 25 μ g bacterial lipopolysaccharide (LPS). Hamsters were housed in either long (LD; panel A) or short (SD; panel B) day lengths for 11 weeks prior to LPS treatment. Injections were delivered between 16:30 and 17:00 in all animals. Note discontinuity along the abscissa from 23:00 h to 01:00 h, during which T_b data are not depicted (see Methods). Black bars along the abscissa indicate the timing of the scotophase. * $p \leq 0.05$ vs. saline-injected group.

Figure 3.

Mean (±SEM) changes in food intake (panel A), body weight (panel B), and sweetened condensed milk intake (expressed as a percentage of baseline values) of adult male Siberian hamsters housed in long (LD) or short day lengths (SD) during the two days (day +1, day +2) immediately following i.p. injection with 100 ng recombinant mouse interleukin-1 beta (rIL-1β). * p ≤ 0.05 vs. saline-injected group in the same photoperiod; # p ≤ 0.05 vs. rIL-1β-treated LD group.

Figure 4.

Mean ($\pm$ SEM) core body temperature (T_b) of adult male Siberian hamsters during the 3 hours prior to, and 24 hours after, i.p. treatment with 100 ng recombinant mouse interleukin-1 beta (rIL-1 β). Hamsters were housed in either long (LD; panel A) or short (SD; panel B) day lengths for 11 weeks prior to rIL-1 β treatment. Injections were delivered between 16:30 and 17:00 in all animals. Note discontinuity along the abscissa from 23:00 h to 01:00 h, during which T_b data are not depicted (see Methods). Black bars along the abscissa indicate the timing of the scotophase. * $p < 0.05$ vs. saline-injected group.

Table 1

Mean ($\pm$ SEM) body mass, estimated testis volumes (ETV), baseline daily food intake, and baseline 6 h ingestion of sweetened condensed milk of photoresponsive Siberian hamsters following exposure to long (LD; 15L:9D) or short (SD; 9L:15D) photoperiods, and immediately prior to injection with saline, lipopolysaccharide, or recombinant interleukin-1 β .

Parameter	LD (n=38)	SD (n=21)
Body mass (g)	36.2 ($\pm$ 0.6)	26.3 ($\pm$ 0.6) *
Estimated testis volume	882 ($\pm$ 23)	191 ($\pm$ 15) *
Food intake (g / 24 h)	3.0 ($\pm$ 0.1)	1.9 ($\pm$ 0.1) *
Sweetened milk intake (g / 6 h)	3.8 ($\pm$ 0.1)	3.0 ($\pm$ 0.1) *

* denotes value differs significantly ($p < 0.05$) from that of long day hamsters.