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Pineal-dependent and -independent effects of photoperiod on immune function in Siberian hamsters (*Phodopus sungorus*)

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Abstract

Siberian hamsters (*Phodopus sungorus*) exhibit reproductive and immunological responses to photoperiod. Short (<10-h light/day) days induce gonadal atrophy, increase leukocyte concentrations, and attenuate thermoregulatory and behavioral responses to infection. Whereas hamster reproductive responses to photoperiod are dependent on pineal melatonin secretion, the role of the pineal in short-day induced changes in immune function is not fully understood. To examine this, adult hamsters were pinealectomized (PINx) or sham-PINx, and transferred to short days (9-h light/day; SD) or kept in their natal long-day (15-h light/day; LD) photoperiod. Intact and PINx hamsters housed in LD maintained large testes over the next 12 weeks; sham-PINx hamsters exhibited gonadal regression in SD, and PINx abolished this effect. Among pineal-intact hamsters, blood samples revealed increases in leukocyte, lymphocyte, CD62L+ lymphocyte, and T cell counts in SD relative to LD; PINx did not affect leukocyte numbers in LD hamsters, but abolished the SD increase in these measures. Hamsters were then treated with bacterial lipopolysaccharide (LPS), which induced thermoregulatory (fever), behavioral (anorexia, reductions in nest building), and somatic (weight loss) sickness responses in all groups. Among pineal-intact hamsters, febrile and behavioral responses to LPS were attenuated in SD relative to LD. PINx did not affect sickness responses to LPS in LD hamsters, but abolished the ameliorating effects of SD on behavioral responses to LPS. Surprisingly, PINx failed to abolish the effect of SD on fever. In common with the reproductive system, PINx induces the LD phenotype in most aspects of the immune system. The pineal gland is required for photoperiodic regulation of circulating leukocytes and neural-immune interactions that mediate select aspects of sickness behaviors.

Keywords

Melatonin; Seasonality; Sickness behaviors; Neural-immune interactions

Introduction

Many non-tropical animals display seasonal morphological, physiological, and behavioral changes in response to changing environmental factors (Bronson, 1989). One of the most salient time-of-year cues is the annual change in day length (photoperiod; Goldman, 2001). Individuals of many mammalian species undergo marked seasonal adaptations when

exposed to changes in photoperiods under laboratory conditions. For example, Siberian hamsters (*Phodopus sungorus*) maintained in short, winter-like photoperiods (e.g., <13 h of light/day for 6 weeks) in the laboratory display decreased body mass and undergo gonadal regression. In the field, photoperiod-induced inhibition of reproductive physiology ensures breeding occurs only during the fraction of the year when energetic resources (food, ambient temperatures) are most permissive (Prendergast et al., 2002a).

Seasonal changes in multiple aspects of immune function have also been described in Siberian hamsters and in several other long-day breeding rodents. The adaptive significance of such changes may lie in the reallocation of energetic resources towards mechanisms necessary for host defense, thereby increasing the likelihood of over winter survival (reviewed in Nelson and Demas, 1996; Nelson, 2004). In Siberian hamsters, short days enhance natural killer cell cytolytic capacity and spontaneous blastogenesis in both whole blood lymphocytes and isolated lymphocytes (Yellon et al., 1999a). Circulating lymphocyte concentrations (e.g., T cells, B cells) are greater in short days relative to long days (Bilbo et al., 2002a; Prendergast et al., 2004a; Yellon et al., 2005). In addition, more integrative measures of immune function such as T-cell-dependent skin inflammatory responses, which depend in part on specific leukocyte subtypes (i.e., CD44+ and CD62+ leukocytes), are greater in short days relative to long days (Dhabhar et al., 2000; Bilbo et al., 2002a; Prendergast et al., 2004a). Short days also ameliorate the acute-phase symptoms of bacterial infection in Siberian hamsters: LPS-induced IL-1 β , IL-6, and TNF- α production are lower (Bilbo et al., 2002b; Prendergast et al., 2003a), and the magnitude and/or persistence of thermoregulatory (fever), behavioral (anorexia), emotional (anhedonia), and ponderal (cachexia) responses to simulated infection are attenuated (Bilbo et al., 2002b).

Understanding how photoperiod information gains access to the neuroendocrine system and the central nervous system remains a major challenge to the study of seasonality. In Siberian hamsters and most other photoperiodic mammals, it is well established that the secretion of pineal melatonin is necessary for the majority of seasonal responses to changes in day length. Entrainment of the circadian system to short days results in an expansion of the duration of the nocturnal pineal melatonin secretion (Illnerova, 1991); long-duration melatonin signals, in turn, act on thalamic and hypothalamic targets to inhibit gonadotrophin secretion (Badura and Goldman, 1992; Bartness et al., 1993; Goldman, 2001). Pinealectomy abolishes reproductive and somatic responses to short days (Hoffman and Reiter, 1965; Yellon and Goldman, 1984; Vitale et al., 1985).

Insights into the dependence of photoperiodic time measurement on pineal melatonin have been derived almost exclusively from studies of photoperiodic changes in reproductive physiology and adiposity. Consequently, little is known about the extent to which photoperiodic changes in immune function rely on the endogenous production of pineal melatonin signals. In one previous experiment, pharmacological injections of melatonin delivered daily 4 h before the onset of darkness (so as to lengthen the endogenous melatonin profile) mimicked the effects of short-days on febrile responses to a simulated infection, suggesting that the duration of melatonin is sufficient in this regard (Bilbo and Nelson, 2002); a similar paradigm yielded comparable results on circulating leukocyte concentrations (Prendergast et al., 2003b). Pineal dependence of innate immunity has been inferred from a study which maintained hamsters in constant light, suppressing endogenous melatonin secretion (Yellon et al., 2005); pineal dependence of adaptive immunity has been inferred from an experiment which exposed pinealectomized hamsters to short days for 1 week prior to antigenic challenge (Yellon et al., 1999b). The interpretation of these studies is not straightforward, given that: (1) daily melatonin injections also render the circadian system in a SD-typical state (Puchalski and Lynch, 1988; Margraf and Lynch, 1993), (2) little is known regarding the significance of the amplitude of melatonin signals on immune

function, and (3) constant light renders circadian rhythms in a free-running state, functions as a stressor, and can be immunosuppressive (Morimoto et al., 1975; Ramaley, 1977; Larsen et al., 1994; Liebmann et al., 1996). An alternative, and definitive, approach is to determine whether photoperiodic responses in the immune system endure following removal of the endogenous pineal melatonin signal (via surgical pinealectomy) and exposure to longer seasonal intervals of short days (>6 weeks).

The purpose of this experiment was to test the hypothesis that photoperiodic changes in enumerative measures of immune function and in behavioral symptoms of simulated infection are dependent on pineal melatonin secretion. Siberian hamsters were surgically pinealectomized or remained pineal-intact and then were exposed to long or short photoperiods for 12 weeks. Circulating leukocytes were enumerated using flow cytometry, after which hamsters were challenged with a simulated bacterial infection to assess thermoregulatory and behavioral acute-phase sickness behaviors. If photoperiodic changes in any aspects of immune function are dependent on pineal-mediated day length signals, then pinealectomy would be predicted to abolish such changes.

Methods

Animals and housing

Male Siberian hamsters (*P. sungorus*) were obtained from a laboratory breeding colony maintained at the University of Chicago. The colony was established from 20 breeding pairs purchased in Spring 2003 from Dr. K. Wynne-Edwards (Queen's University, Kingston, Ontario, Canada) which has been maintained in captivity since 1988, but is regularly out-bred with wild-caught stock. Hamster pups were weaned at 18–21 days of age and housed 2–4 per cage with same-sex siblings in polypropylene cages (28×17×12 cm) with wood shaving beddings (Harlan Sani-Chips, Harlan Inc., Indianapolis, IN, USA) in a 15L:9D light–dark cycle (lights off at 1800 h CST) until 84–104 days (3–4 month) of age. Ambient temperature of the room was 20±0.5 °C and relative humidity was maintained at 53±2%. Food (Teklad Rodent Diet 8604, Harlan Inc.) and filtered tap water were provided *ad libitum*. Cotton nesting material was constantly available in the cage until aliquoted for nest-building tests after *week 12* (see below). All procedures were conformed to the USDA Guidelines for the Care and Use of Laboratory Animals and the Institutional Animal Care and Use Committee (IACUC) of the University of Chicago.

Surgical procedures

At 70–90 days of age, hamsters were surgically pinealectomized (PINx, *n*=21) or sham-operated (Sham, *n*=20) under sodium pentobarbital (0.05 mg/g, Nembutal, Abbott Laboratories, North Chicago, IL, USA) anesthesia according to surgical procedures described in Bartness and Goldman (1988). In brief, the head was shaved and a rostrocaudal incision of approximately 2 cm was made through the skin along the midline. Connective tissues were cleared, and a small oval piece (~3 mm in diameter) of skull surrounding lambda was removed using a hand-held drill. The pineal gland was removed with a pair of micro-dissecting forceps and immediately verified under a 20× dissecting scope. The opening in the skull was sealed with gelfoam, and the skin was closed using stainless steel wound clips. Sham-operated hamsters were subjected to an identical procedure except the dissecting forceps were never inserted. After surgery, hamsters received analgesia (0.5 µg/g, s.c., Buprenex, Reckitt Benckiser Pharmaceuticals, Richmond, VA, USA) twice per day for 2 days.

Photoperiod manipulations

After 2 weeks of recovery (= *week 0*), hamsters from each surgical treatment group were transferred either into short days (9-h light/day SD, $n=11$ PINx, $n=10$ Sham-PINx) (lights on at 0900 h CST) or remained in long days (15-h light/day LD; $n=10$ PINx, $n=10$ Sham-PINx). Photoperiodic responsiveness was determined from changes in body mass, estimated testis volume, and fur molt as measured at *week 10*. Testis size was estimated by measuring the length and width of the left testis through the abdominal skin while under light isoflurane anesthesia. This provides a measure of estimated testis volume (ETV), which is correlated with testis weight, circulating testosterone and spermatogenesis (Gorman and Zucker, 1995; Schlatt et al., 1995).

Immune measurements

Flow cytometry—At *week 10*, blood samples (approximately 270 μ l) for flow cytometry were drawn under light anesthesia (between 1245 h and 1315 h, 5 h prior to lights off) from the right retro-orbital sinus using heparinized Natelson collection tubes and dispensed into vials loaded with 30 μ l heparin (30 units). To minimize stress, blood collections were performed in a room separate from the general animal colonies, and following the procedure, hamsters were separated from the colony until all blood collections for the day were completed. Animals were quickly and gently placed into an anesthesia chamber (pre-saturated with 5% isoflurane) by hand. The duration of time required to obtain a blood sample, from the initiation of anesthesia to the filling of the blood collection tube, was approximately 1 min. Following blood collection, hamsters were administered 0.5 ml sterile 0.9% saline s.c. for rehydration. Whole blood samples were kept at room temperature and analyzed by flow cytometry within 24 h. Lymphocyte, neutrophil, and monocyte subpopulations were identified and gated by using forward- versus side-scatter characteristics. T cells were identified by using CyChr-labeled anti-CD3 (clone 145-2C11). Neutrophils and monocytes were identified by using forward- versus side-scatter patterns. L-selectin and CD44-positive cells were identified by using phycoerythrin-labeled anti-CD62L (MEL-14) and FITC labeled anti-CD44 (IM7), respectively. Each staining panel consisted of a single Ab. All monoclonals were directly conjugated, rat anti-mouse Abs (except for NK1.1, a murine Ab) and were obtained from Becton Dickinson-PharMingen (San Jose, CA, USA).

Sickness behaviors—Two weeks after blood collection (*week 12*), daily body mass and food intake measurements (performed at 1730 h, 30 min before the onset of darkness) were initiated. After 3 days of baseline measurements, shortly before lights off (1730–1800 h), hamsters were injected i.p. with either bacterial lipopolysaccharide (LPS, 25 μ g/hamster; isolated from *E. coli* strain 026:B6, Lot 064K4077, Sigma, St. Louis, MO) or sterile 0.9% saline (0.1 ml) in a counter-balanced design, with successive injections separated by 8 days. On the night of injection, baseline body temperatures were collected immediately before injections and 2, 4, 6, 8, 14, and 18 h after injection, using a Thermalert TH-5 thermometer with a lubricated thermocouple rectal probe (Physitemp, Clifton, NJ). At the time of injection, each hamster was given a small piece of cotton batting (~3 g), which was weighed before and again 6 h after presentation, in order to assess nesting material use. Hamsters typically shred this cotton to construct a small nest within the first few hours after the offering.

Statistical analyses

Body mass and ETV values obtained at *week 0* and *week 10* were compared using ANOVA. Because photoperiod significantly affected body mass and ingestive behavior prior to LPS administration (see results), changes in body mass and food 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)×2 (PINx, Sham)×2 (LPS, Saline) factorial ANOVA, according to the methods of Bilbo et al. (2002b). Data collected between the time of LPS/saline treatment and 24 h later are designated as “day+1” values, those collected between 24 h and 48 h after injection are referred to as “day+2” values, and so on. Where permitted by a significant omnibus F value, pairwise comparisons were conducted using t -tests.

The effect of the injection treatment order on change in body mass, anorexia, and nest building was evaluated with ANOVA. No significant differences were found between orders of injection treatment ($p>0.05$ in all comparisons), and injection groups were collapsed within treatment across the counterbalanced blocks.

Changes in colonic temperature (T_c) were compared separately within each photoperiod using repeated measures ANOVA. Pairwise comparisons between injection groups at individual time points were conducted using t -tests. LPS injections result in sustained (>4–6 h) febrile responses (cf. Bilbo et al., 2002b). 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_c in LPS-injected hamsters were established based on the mean and variance in T_c of saline-injected hamsters. T_c was considered elevated when it exceeded the 95% confidence limits of the mean T_c value of saline-injected hamsters at any given measurement time point. To avoid labeling transient fluctuations in T_c as meaningful elevations, only T_c values which together comprised a sustained increase in T_c (defined as >3 successive readings above the 95% confidence limit) were regarded as meaningful elevations in T_c . For each animal, the beginning of the first sustained increase in T_c following injection marked the onset of the febrile response, and the total amount of elevated T_c during the 18 h following injection defined the duration of the febrile response. The duration of fever was compared between groups using t -tests.

All statistical calculations were conducted using Statview 5.0 (SAS Institute, Cary, NC). Differences were considered statistically significant if $p<0.05$.

Results

Somatic and reproductive responses

There was a significant effect of photoperiod ($F_{1,37}=27.7$, $p<0.001$) and a significant interaction between photoperiod and surgery ($F_{1,37}=21.5$, $p<0.001$) on *week 10* body mass. Pineal-intact hamsters housed in SD had significantly lower body masses ($t_{20}=7.00$, $p<0.001$) than hamsters housed in LD. In contrast, PINx hamsters in LD and SD had comparable body masses ($t_{17}=0.45$, $p>0.05$; Fig. 1A). Likewise there was a significant effect of photoperiod ($F_{1,37}=165.6$, $p<0.001$) and a interaction between photoperiod and surgery ($F_{1,37}=167.1$, $p<0.001$) on *week 10* ETV. Pineal-intact hamsters housed in SD had significantly smaller testis sizes ($t_{20}=19.2$, $p<0.001$) than hamsters housed in LD. PINx hamsters in SD had ETVs comparable to those of LD PINx hamsters ($t_{17}=0.04$, $p>0.05$) and significantly greater than those of SD pineal-intact animals ($t_{19}=24.7$, $p<0.001$; Fig. 1B).

Leukocyte enumeration

There was a significant effect of photoperiod on several measures of circulating leukocytes. Among pineal-intact hamsters, the concentration of total circulating leukocytes (Fig. 2A), total lymphocytes (Fig. 2B), CD62L+ lymphocytes (Fig. 2C), and CD3+ T lymphocytes (Fig. 2D) were each significantly higher in SD as compared to LD (t_{20} 4.52, $p<0.05$ in all cases). Among PINx hamsters, these photoperiodic differences in cell counts were abolished (t_{17} 1.80, $p>0.05$ in all cases; Figs. 2A–D). Neither pinealectomized nor pineal-intact

hamsters exhibited differences in counts of circulating monocytes, neutrophils, CD62L+ monocytes, or CD62L+ neutrophils between LD and SD (data not shown).

Sickness behaviors

Among pineal-intact hamsters, there was a significant effect of injection ($F_{1,40}=5.20$, $p<0.05$) and an interaction between injection and photoperiod ($F_{1,40}=13.7$, $p<0.001$) on change in food intake following injection treatments (Fig. 3A). Food intake was significantly decreased in hamsters injected with LPS compared to those treated with saline; this decrease persisted for 3 days following LPS injection in LD hamsters, as compared to 1 day in SD hamsters (t_{18-22} 2.18, $p<0.05$ in all cases). The peak magnitude of the decrease in food intake was also significantly greater in LD (~80% decrease) relative to SD (<50% decrease) (t_{20} = 3.92, $p<0.001$).

In PINx hamsters, LPS treatments suppressed food intake relative to baseline values ($F_{1,34}=22.5$, $p<0.001$), but the effect of LPS did not differ as a function of photoperiod ($F_{1,34}=1.38$, $p>0.05$; Fig. 3B). LPS-induced anorexia was significant as compared to saline on the first 3 days following injection (t_{16-18} 2.41; $p<0.05$ in all cases), regardless of photoperiod. The peak magnitude decrease in food intake was also comparable between LD and SD PINx hamsters (~80%; $t_{17}=1.53$, $p>0.05$ in all cases).

Body mass loss

Among pineal-intact hamsters, there was a significant effect of injection ($F_{1,40}=17.3$, $p<0.001$) and an interaction between injection and photoperiod ($F_{1,40}=5.00$, $p<0.05$) on change in body mass following injections (Fig. 4A). LD hamsters lost a significant percentage of their body mass after LPS as compared to saline injection, and this decrease persisted throughout the entire 6 day post-injection measurement interval (t_{18} 3.26, $p<0.005$ in all cases). Mean body mass in SD hamsters decreased slightly after LPS injection, but never differed significantly from that of saline-injected SD controls ($t_{22}<1.75$, $p>0.05$ in all cases). Compared to SD hamsters, hamsters in LD lost a significantly greater percentage of their body mass following LPS treatments (t_{20} 3.34, $p<0.005$ in all cases).

In PINx hamsters, there was a significant effect of injection treatment on change in body mass ($F_{1,34}=24.8$, $p<0.001$), but this effect did not vary as a function of photoperiod ($F_{1,34}=0.275$, $p>0.05$) (Fig. 4B). PINx hamsters lost significantly more body mass after LPS relative to saline injection (LD: t_{18} 2.25, $p<0.05$ in all cases; SD: t_{16} 2.22, $p<0.05$ in all cases), but this body mass loss was comparable among LD and SD hamsters ($t_{17}<1.71$, $p>0.05$ in all cases).

Nest-building behavior

Among pineal-intact hamsters, there was a significant effect of injection ($F_{1,40}=7.03$, $p<0.05$) and a significant interaction between injection and photoperiod ($F_{1,40}=4.99$, $p<0.05$) on the amount of nesting material used during the 6 h after injection treatments. In LD, hamsters shredded significantly less nesting material after LPS relative to saline treatment ($t_{18}=2.69$, $p<0.05$); whereas in SD hamsters, LPS treatment failed to suppress nesting material use ($t_{22}=0.41$, $p>0.05$; Fig. 5). Among PINx hamsters, LPS treatments significantly suppressed nesting material use ($F_{1,34}=6.74$, $p<0.05$), but this effect was comparable ($F_{1,34}=0.04$, $p>0.05$) in LD and SD (LD: $t_{18}=2.81$, $p<0.05$; SD: $t_{16}=2.17$, $p<0.05$; Fig. 5).

Febrile responses

Among pineal-intact hamsters, LPS injection significantly altered the pattern of colonic temperature (T_c) relative to that of saline-treated controls in both LD ($F_{7,126}=2.10$, $p<0.05$) and SD ($F_{7,154}=7.95$, $p<0.001$). LD hamsters injected with LPS had significantly higher T_c

at 2, 4, 12, and 14 h after injections compared to their respective saline-injected controls ($t_{18} = 2.78$, $p < 0.05$ in all cases). In contrast, SD hamsters had significantly higher T_c only at 2 and 4 h after LPS injection ($t_{22} = 3.85$, $p < 0.05$ in both cases; Fig. 6A). Among PINx hamsters, LPS injection likewise significantly altered the pattern of T_c relative to saline-treated controls both in LD ($F_{7,126} = 3.89$, $p < 0.001$) and in SD ($F_{7,112} = 4.65$, $p < 0.001$). LD hamsters had significantly higher T_c at 2, 4, 6, and 12 h after LPS injection compared to their saline-treated controls ($t_{18} = 2.74$, $p < 0.05$ in all cases), and SD hamsters had significantly higher T_c at 2 h after LPS injection ($t_{16} = 3.39$, $p < 0.05$; Fig. 6B). All LPS-injected hamsters had comparable patterns of T_c ($F_{3,37} = 0.89$, $p > 0.05$).

Among LPS-injected hamsters, there was a significant effect of photoperiod on fever duration ($F_{1,37} = 11.1$, $p < 0.005$), but the nature of this effect did not differ as a function of pineal status ($F_{1,37} = 0.104$, $p > 0.05$). Mean fever duration was significantly longer in LD relative to SD hamsters in both pineal-intact ($t_{20} = 2.19$, $p < 0.05$) and PINx hamsters ($t_{17} = 2.54$, $p < 0.05$). Within each photoperiod condition, fever duration was also comparable between pineal-intact and PINx hamsters (LD: $t_{18} = 0.77$, $p > 0.05$; SD: $t_{19} = 0.58$, $p > 0.05$; Figs. 6A, 6B).

Discussion

Following adaptation to short photoperiods, Siberian hamsters increased the concentrations of total leukocytes and of several lymphocyte subtypes in the general circulation. Exposure to SD also attenuated both the magnitude and the duration of two major consequences of bacterial infection (anorexia and cachexia), replicating earlier work in this species (Bilbo et al., 2002b; Prendergast et al., 2004b) which demonstrated that exposure to SD enhances the number of circulating leukocytes (and thus capacity for immunosurveillance) and mitigates symptoms of infection. The present report also extends the repertoire of sickness behaviors affected by photoperiod to include thermoregulatory behaviors associated with the use of nest building material. Surgical pinealectomy abolished the effects of short photoperiods on circulating leukocyte counts and on the behavioral and somatic sickness responses to LPS. This outcome directly implicates the pineal gland, and most likely its chief secretory product, melatonin, as necessary for the expression of peripheral immune responses to short photoperiods and for the instantiation of photoperiodic changes in neural-immune interactions that govern sickness behaviors. Thus, in common with the reproductive system, the immune system relies on photoperiodic time measurement mechanisms that include the pineal gland to engage photoperiodic changes in physiological and behavioral components of immune function.

Photoperiodic differences were also evident in the duration of fever following simulated infection. Although the present data replicate the short-day attenuation of febrile responses after LPS challenge (Bilbo et al., 2002b), photoperiodic changes in the nature of the febrile response to LPS did not require an intact pineal gland. Febrile responses instead appeared largely driven by the circadian rhythm in body temperature, which was differently phased in hamsters housed in LD relative to SD. Indeed, absolute T_c values of LPS-treated hamsters were similar among LD and SD groups. The protracted febrile responses to LPS observed in LD hamsters appeared to arise largely from the earlier T_c decline associated with the onset of the light phase in saline-treated LD hamsters. This suggests that the photoperiodic differences in fever duration between LD and SD reported here and elsewhere (Bilbo and Nelson, 2002; Bilbo et al., 2002b) may be due in part to entrainment of the circadian rhythm in body temperature. Given that the generation of circadian rhythms in the periphery is driven by the neural and humoral output of the SCN (Silver et al., 1996; Meyer-Bernstein et al., 1999; Kramer et al., 2001), and is almost entirely independent of any reciprocal influence by the pineal gland or melatonin (Sumova and Illnerova, 1996; Prendergast and

Freeman, 1999), PINx would be expected to have little effect on photoperiodic differences in the febrile response to LPS if such differences are due solely to circadian factors.

Total leukocyte counts served as an omnibus indicator of whether photoperiod was registered by the immune system following the experimental manipulations (day length and PINx). Among pineal-intact hamsters, paralleling the SD-induced increases in total leukocytes were significant increases in the number of lymphocytes, T cells, and CD62⁺ leukocytes. Increases in the number of cells expressing CD62 cell surface markers – adhesion molecules that participate in the migration of lymphocytes through endothelium during inflammatory responses – together with increases in total T cells, would be predicted to facilitate delayed-type hypersensitivity (DTH) inflammatory responses in SD (Chen et al., 1997; Brocke et al., 1999), as has been reported previously (Bilbo et al., 2002a,b; Prendergast et al., 2004a; 2005). The elimination of photoperiodic enhancement of T cells and CD62⁺ leukocytes by PINx suggests that SD enhancement of such inflammatory responses would likely be abolished by PINx as well.

Similar to its role in the reproductive and somatic responses to photoperiod, the present data suggest that the pineal gland also plays a prominent role in orchestrating photoperiodic changes in peripheral measures of immunity (blood leukocytes), and in changes among neural-immune interactions that participate in sickness behaviors. The pineal gland transduces day length information into a neuroendocrine signal in the form of nocturnal melatonin secretion (Bartness et al., 1993). Long-duration (8 h/night) melatonin signals inhibit the reproductive system, and short-duration signals (6 h/night) stimulate, or permit, reproductive development (Carter and Goldman, 1983a, 1983b). PINx abolishes circulating melatonin concentrations and consequently eliminates any modulation of the reproductive system in response to changes in day length (Yellon and Goldman, 1984). The present data suggest that a common, pineal-dependent mechanism mediates the effects of photoperiod on the immune and reproductive systems. It is tempting to speculate that long-duration melatonin signals are the relevant pineal output for inducing SD-like changes in immune function in neurologically intact hamsters, although such a conclusion awaits the outcome of experiments that use timed daily infusions of melatonin to provoke immunological changes in PINx hamsters (cf. Bartness et al., 1993). From an information-processing perspective, photic regulation of the immune and reproductive systems appears to be governed, at least in part, by a common mechanism, which converges at the output of the pineal gland.

Yet another commonality between the reproductive and immunological processing of pineal-mediated seasonal time cues (or absence thereof) was evident in the manner in which PINx abolished immune responses to photoperiod. PINx abolished photoperiodic regulation of most measures of immune function by rendering them in a LD-like, rather than a SD-like, phenotype. PINx likewise renders the reproductive system of Syrian (Hoffman and Reiter, 1965) and Siberian (Carter and Goldman, 1983a) hamsters in a state of permanent reproductive competence (i.e., in the LD-phenotype). This observation suggests that the absence of a melatonin signal is likewise encoded by the immune system either as a LD signal, or as permissive for the expression of prior photoperiod history effects (Anchordoguy and Lynch, 2000; Prendergast et al., 2000, 2002a).

The immune system and the reproductive system may independently gain access to pineal-mediated day length information (i.e., melatonin signals); alternatively, immune responses to photoperiod may depend on the concurrent reproductive responses. In the latter scenario, the effect of PINx on photoperiodic changes in the immune system may be a result of the elimination of reproductive responses to photoperiod. Thus, PINx rendered SD animals in a LD-like immune status because the procedure rendered animals in LD-like reproductive condition. Indeed, gonadal androgens influence immune function—often in an

immunosuppressive fashion (Klein, 2000; Obendorf and Patchev, 2004; Muehlenbein and Bribiescas, 2005). However, in Siberian hamsters, several experiments have indicated that photoperiodic changes in immune function can occur independent of changes in reproductive system (blastogenesis: Prendergast et al., 2002b; leukocyte counts: Prendergast et al., 2004a; skin inflammatory responses: Prendergast et al., 2005). Studies in other reproductively photoperiodic species have indicated similar gonadal hormone-independent effects of photoperiod on the immune system (Demas and Nelson, 1998). The extent to which changes in neural-immune interactions that participate in photoperiodic changes in sickness behaviors depend on photoperiodic changes in gonadal hormone secretion is presently not fully understood.

If the effects of PINx are not manifest simply through the elimination of reproductive responses, then the present data would be compatible with a separate, possibly direct, action of pineal melatonin on targets within the immune system. The sites of these putative actions are not known, but may involve central (Badura and Goldman, 1992; Freeman and Zucker, 2001; Demas et al., 2002, 2003) or peripheral (Maestroni, 1998; Drazen et al., 2000; Prendergast et al., 2001; Kriegsfeld et al., 2001; Hotchkiss and Nelson, 2002) tissues. The sites of melatonin action (central versus peripheral) and the manner in which such action contributes to the photoperiodic changes in immune function and sickness behaviors reported here, awaits further study.

From a broader ecological perspective, our data are congruent with the winter immunoenhancement hypothesis (Nelson and Demas, 1996), which emphasizes a prominent role of energy balance in the evolution of photoperiodic seasonal changes in immune function. The ability of LPS to induce sickness behaviors that impinge on energy balance appears diminished in hamsters housed in short photoperiods. Anorexia and fever both deplete energy stores, thus during winter, animals may benefit from suppressing the magnitude and duration of such energetically expensive responses during a time when food and resources are scarce. Nest building is likewise a behavioral response that contributes to thermoregulatory capacity by reducing energy expenditure in cold environments (Kauffman et al., 2003). These data indicate that adaptation to SD protects hamsters from the suppressive effect of illness on this potentially important thermoregulatory behavior.

In summary, hamsters housed in short photoperiods exhibited increases in circulating leukocytes and diminished acute phase symptoms of bacterial infection. Such photoperiodic changes in peripheral immunity and neural-immune interactions were abolished by PINx, illustrating their pineal dependence. Photoperiodic differences in febrile responses to LPS were still evident following PINx. Thus, the role of the pineal gland in photoperiodic adjustments of integrated aspects of immune function following infection appears to be trait-specific.

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Fig. 1. (A) Mean ($\pm$ SEM) body mass, and (B) estimated testis volume ($\pm$ SEM) of adult male Siberian hamsters that were either pinealectomized (PINx) or sham-operated (Sham-PINx), and transferred to either long-day (LD, 15-h light/day) or short-day (SD, 9-h light/day) photoperiods for 10+weeks. * $p < 0.05$ versus all other groups.

Fig. 2.

Mean (+SEM) concentrations of (A) total leukocytes, (B) total lymphocytes, (C) CD62L +leukocytes, and (D) CD3+T cells in whole blood of pinealectomized (PINx) and intact (Sham-PINx) Siberian hamsters following 10 weeks of exposure to either long (LD) or short (SD) days. * $p < 0.05$ versus LD value within a surgical treatment group.

Fig. 3.

Mean ($\pm$ SEM) percent change in daily food intake from baseline in adult male Siberian hamsters following i.p. injection with 25 μ g of bacterial lipopolysaccharide (LPS) or sterile 0.9% saline (Saline; injections delivered on *day 0*). Prior to injection treatments, hamsters were either (A) sham-operated (Sham) or (B) pinealectomized (PINx) and then housed in either long days (LD) or short days (SD) for 12–13 weeks. Within each panel: * $p < 0.05$ versus Saline-injected group exposed to the same photoperiod; # $p < 0.05$ versus SD LPS-treated group.

Fig. 4. Mean ($\pm$ SEM) percent change in body mass from baseline in (A) sham-operated and (B) PINx male Siberian hamsters following LPS and Saline treatments. Photoperiod, surgical, and injection treatments as described in Fig. 3. Within each panel: * p <0.05 versus Saline-injected group exposed to the same photoperiod; # p <0.05 versus SD LPS-treated group.

Fig. 5. Mean (+SEM) nesting material use in (A) sham-operated and (B) PINx male Siberian hamsters following LPS and Saline treatments. Photoperiod, surgical, and injection treatments as described in Fig. 3. * $p < 0.05$ versus Saline-treated group exposed to the same photoperiod and surgical treatments; # $p < 0.05$ versus Sham-operated, LPS-treated SD value.

Fig. 6. Mean ($\pm$ SEM) colonic temperature (T_c) of (A) sham-operated and (B) PINx male Siberian hamsters following LPS and Saline treatments. Photoperiod, surgical and injection treatments as described in Fig. 3. At the bottom of each panel is depicted fever duration (see Methods) for LPS-treated animals only. Within each panel: * $p < 0.05$ versus Saline-treated group exposed to the same photoperiod and surgical treatments; # $p < 0.05$ versus LD value.