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Winter Day Lengths Enhance T Lymphocyte Phenotypes, Inhibit Cytokine Responses, and Attenuate Behavioral Symptoms of Infection in Laboratory Rats

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Abstract

Annual variations in day length (photoperiod) trigger changes in the immune and reproductive system of seasonally-breeding animals. The purpose of this study was to determine whether photoperiodic changes in immunity depend on concurrent photoperiodic responses in the reproductive system, or whether immunological responses to photoperiod occur independent of reproductive responses. Here we report photoperiodic changes in enumerative, functional, and behavioral aspects of the immune system, and in immunomodulatory glucocorticoid secretion, in reproductively non-photoperiodic Wistar rats. T-cell numbers (CD3+, CD8+, CD8+CD25+, CD4+CD25+) were higher in the blood of rats housed in short as opposed to long day lengths for 10 weeks. Following a simulated bacterial infection (*E. coli* LPS; 125 μg/kg) the severity of several acute-phase sickness behaviors (anorexia, cachexia, neophobia, and social withdrawal) were attenuated in short days. LPS-stimulated IL-1β and IL-6 production were comparable between photoperiods, but plasma TNFα was higher in long-day relative to short-day rats. In addition, corticosterone concentrations were higher in short-day relative to long-day rats. The data are consistent with the hypothesis that photoperiodic regulation of the immune system can occur entirely independently of photoperiodic regulation of the reproductive system. In the absence of concurrent reproductive responses, short days increase the numbers of leukocytes capable of immunosurveillance and inhibition of inflammatory responses, increase proinflammatory cytokine production, increase immunomodulatory glucocorticoid secretion, and ultimately attenuate behavioral responses to infection. Seasonal changes in the host immune system, endocrine system, and behavior may contribute to the seasonal variability in disease outcomes, even in reproductively non-photoperiodic mammals.

Keywords

photoperiodism; sickness behavior; depression; IL-1β; IL-6; TNFα; corticosterone

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Introduction

Winter presents animals with a significant energetic challenge: food availability is scarce and the costs of thermoregulation are higher, relative to conditions during spring and summer (Bronson, 1989). To contend with this energetic conflict, many mammals have evolved mechanisms which allow them to suspend reproduction during the fraction of the year when environmental conditions are unfavorable for successful weaning of offspring (Prendergast et al., 2002a; Schneider, 2004). The primary cue for engaging these changes in reproductive physiology is the seasonal change in day length (photoperiod), which reliably predicts winter conditions weeks in advance of their arrival. In many long-day breeding mammals, decreasing photoperiods of late-summer induce gonadal atrophy, decreases in gonadal hormone secretion, and termination of sex behaviors (Prendergast et al., 2002a). In the laboratory, this entire suite of changes in the reproductive system can be induced by transfer of reproductively photoperiodic animals from long, summer-like photoperiods (typically those in excess of 14 h of light per day) to short winter-like photoperiods (less than 10 h of light per day; Goldman, 2001)

Photoperiodic changes in immune function are also evident in wild and laboratory animals (Nelson, 2004), but relative to those operant in the reproductive system, the central and peripheral mechanisms by which photoperiodic changes in the immune system are accomplished are less-well known. In general, among long-day breeding rodents, most, though not all (Drazen et al., 2000; Kinsey et al., 2003), aspects of immune function are enhanced under reproductively inhibitory short days. A prevailing hypothesis is that, during winter, animals reallocate energy away from reproduction and toward maintenance of host defense (Nelson, 2004). For example, deer mice (*Peromyscus maniculatus*) exhibit increases in specific antibody production (Demas and Nelson, 1996) and lymphocyte antigen-responsiveness (Demas and Nelson, 1998a) following exposure to several weeks of short days; these changes in immune function occur in parallel with the cessation of reproductive activity (Desjardins et al., 1986). Siberian hamsters (*Phodopus sungorus*) also exhibit a host of changes in immune function following adaptation to short photoperiods: antibody production is decreased, but circulating leukocyte, T-cell and B-cell counts are greater, natural killer cell cytotoxicity is facilitated, and antigen-specific skin inflammatory responses are enhanced (Drazen et al., 2000; Bilbo et al., 2002a; Yellon at al., 1999). Short days also ameliorate the symptoms of bacterial infection: LPS-induced production of IL-1β, IL-6, and TNFα is lower in short days (Bilbo et al., 2002b; Prendergast et al., 2003a), and the magnitude and/or persistence of thermoregulatory, behavioral, emotional, and ponderal responses to infection are markedly attenuated (Bilbo et al., 2002b). These changes likewise parallel the regression of the reproductive system.

The majority of research on photoperiodic regulation of the immune system has been undertaken in reproductively photoperiodic animals, primarily Siberian hamsters and deer mice —animals in which photoperiod-induced changes in immune function occur concurrently with photoperiod-induced changes in reproductive physiology and gonadal hormone secretion. In light of the potent immunomodulatory role of gonadal hormones in hamsters (Bilbo and Nelson, 2001; Prendergast et al., 2003b) and other mammals (Ansar Ahmed et al., 1985; Klein, 2000), it is possible that photoperiodic changes in immunity arise primarily as a downstream consequence of photoperiodic changes in the reproductive axis. Empirical data on this issue have not yielded consistent results. In Siberian hamsters, photoperiodic regulation of some indices of immunity occurs independent of changes in reproductive physiology and gonadal hormone secretion (antibody production, Drazen et al., 2000; skin inflammatory responses, Prendergast et al., 2005), whereas others appear to track gonadal status (circulating leukocyte concentrations, Prendergast et al. 2003b; spontaneous blastogenesis, Prendergast et al., 2002b). In deer mice, immune responses to changes in photoperiod are absent in subspecies

that fail to exhibit reproductive responses to changes in day length, suggesting a linkage between reproductive and immunological photoresponsiveness (Demas et al., 1996); however, within a given reproductively photoresponsive deer mouse species, *in vivo* measures of lymphocyte responsiveness to mitogens are enhanced in short days, independent of gonadal hormone manipulations (Demas and Nelson, 1998b). Determining the extent to which photoperiodic changes in the immune system depend on activation and inhibition of the reproductive axis remains a key step towards understanding how changes in day length gain access to the immune system, and more generally, stands to provide basic insights into mechanisms by which the activity of the central nervous system impinges on the immune system.

Surgical manipulations of reproductive condition in photoperiodic rodents are but one method of experimental control over gonadal and endocrine status; a convergent approach to this question may be afforded by taking advantage of the heterogeneity of reproductive responsiveness to photoperiod evident among different strains of laboratory animals. In contrast to the outbred non-tropical rodents described above, many laboratory mouse (*Mus musculus*) and rat (*Rattus norvegicus*) strains are reproductively non-photoperiodic, although they possess the neuroendocrine mechanisms sufficient for perceiving changes in day length and for transducing ambient photoperiod into a neuroendocrine melatonin signal (Illnerova and Vanecek, 1980; Nelson et al., 1994; Nelson and Zucker, 1981; Wallen and Turek, 1981). Reproductively non-photoperiodic rats and mice thus may provide useful models to investigate mechanisms by which day length influences the immune system in a manner uncomplicated by photoperiodic influences on the reproductive system. However, evidence for immunological photoperiodism in such species is sparse. In C3H/HeN mice, modest changes in circulating lymphocyte counts were evident following transfer from long to short days, but these enumerative differences did not extrapolate to cell-specific functional assays (Yellon and Tran, 2002). Consistent with this report, both C3H/HeN and C57/BL6 mice exhibited comparable lymphocyte counts and skin inflammatory responses in long and short days (Gatien et al., 2004). Photoperiod likewise failed to affect several *in vitro* measures of T-cell responsiveness in male C57 BL/6J mice (Bhat et al., 2003). Although certain rat strains exhibit changes in immune organ weight and cellularity following extreme manipulations of photoperiod (Mahmoud et al., 1994), and some data suggests low-amplitude circannual rhythms in mammary tumor susceptibility exist in rats (Loscher et al., 1997), to date no study has investigated whether seasonal changes in day length regulate the immune system of laboratory rats of any strain. Moreover, no attempts have been made to identify the mechanisms by which any such seasonal regulation might occur.

The goal of these experiments was to determine whether aspects of immune system that are reported to be under photoperiodic control in reproductively photoperiodic rodents (enumerative aspects of the immune system and behavioral responses to infection) are modulated by photoperiod in species that do not exhibit reproductive responses to photoperiod. Male Wistar rats were selected as a model species because this strain does not exhibit reproductive responses to photoperiod (Vanecek and Illnerova, 1982; Wallen et al., 1987) and is one of the most commonly-used mammals for studies of peripheral immune function and behavioral responsiveness to infection. Any photoperiodic modulation of immunity, immunomodulatory neuroendocrine activity, or behavioral responsiveness to immune activation in this species would constitute evidence that reproductive and immunological responses to day length are not obligately linked.

Methods and Materials

Animals and General Housing Conditions

Male Wistar rats (HsdRccHan:WIST) weighing 225–250 g (53–58 days of age) were purchased from Harlan (Indianapolis, IN, USA) and used in these experiments. After arrival at the University of Chicago vivarium, all rats were housed in randomly-assigned pairs in polypropylene cages (25.9 cm × 47.6 cm × 20.9 cm high) in a temperature-controlled room (22±1°C) under a light-dark cycle that provided 12 h of light and 12 h of darkness per day (12L:12D; lights off at 17:00 CST). During the scotophase, a dim (<1 lux) red light remained on to facilitate experimental procedures conducted in the dark. In all experiments, rats had *ad libitum* access to food (Teklad 8640; Harlan, Indianapolis, IN, USA) and filtered tap water. After one week of habituation to the laboratory environment the experimental manipulations described below began. Three experiments were carried out to determine the effects of changes in day length on different aspects of the immune system and neural-immune interactions.

All procedures were conducted in accordance with the *Guide for Care and Use of Laboratory Animals* established by the National Institute of Health, conforming to AAALAC standards, and was approved by the local Institutional Animal Care and Use Committee.

Experiment 1: Effects of photoperiod on circulating leukocytes—One week after arrival in the laboratory (= *week 0*), 32 male rats were transferred to light-tight chambers (8 cages per chamber) that provided either 16 h light per day (16L:8D; LD; n=16) or 8 h light per day (8L:16D; SD; n=16); the time of lights-off was the same (17:00 h CST) for all photoperiods. The 16L and 8L photoperiods were chosen because they match or exceed in duration those photoperiods sufficient to elicit phenotypic changes in the hamster reproductive and immune systems (Prendergast et al., 2002a; Nelson et al., 2002). Rats were weighed (± 1 g) at weekly intervals for the next 10 weeks. On week 10 between 15:00 – 17:00 h CST, rats were anesthetized using a mixture of 3% isoflurane and medical oxygen at a flow rate of 3 liters/min, and 1 ml of whole blood was obtained via the right retro-orbital sinus using sterilized Pasteur pipettes coated with sodium heparin. Blood samples were thoroughly mixed with 100 μl sodium heparin (100 units) and kept at room temperature until leukocytes were extracted (see *Leukocyte Analyses and Flow Cytometry*, below). Immediately after bleeding, rats were administered 1 ml of sterile 0.9% physiological saline s.c. to restore fluids, and were returned to their photoperiod housing chambers. All bleeding was performed in a room that was acoustically isolated from the housing chambers.

Leukocyte Analyses and Flow Cytometry: Total leukocyte numbers were obtained from whole blood samples according to methods previously reported (Prendergast et al., 2003b): a 25 μl aliquot of the whole blood sample was mixed thoroughly with 3% acetic acid at a 1:20 dilution (Unopette, Beckton-Dickinson) and counted in duplicate on a hemacytometer. In the remainder of the whole blood sample, specific leukocyte subtypes were measured by immunofluorescent antibody (Ab) staining and analysis by using single- and multiple-color flow cytometry (CyanADP, DakoCytomation). Blood was subjected to erythrocyte lysis using an ammonium chloride lysis buffer (155 mM NH_4Cl, 10mM $KHCO_3$, 0.1 mM dsEDTA, pH = 7.3; Pelegri et al., 1995). Briefly, 12 ml of ice-cold lysis buffer was added to 800 ul whole blood and gently inverted for exactly 3 min. The mixture was centrifuged at 300 × *g* for 7 minutes, after which cells were washed twice with phosphate-buffered saline (PBS), resuspended in 4 ml supplemented PBS containing 2% fetal calf serum and 0.1% NaN_3 (sPBS), and held at 4°C. Before staining, the concentration and viability of cell suspensions were assessed using trypan blue exclusion (Pelegri et al., 1995). Mononuclear cells (1.5×10^5) were incubated in 100 ul of monoclonal antibody (at appropriate concentrations as determined by titration, see *Antibodies*, below) for 30 min at 20°C, after which stained cells were washed

twice in PBS, resuspended in 200 ul sPBS buffer, fixed with 2% paraformaldehyde, and stored at 4°C in the dark until analysis.

Antibodies: Before the experiment, appropriate dilutions of each monoclonal antibody (MAb) were established to use saturating antibody concentrations for 1.5×10^5 cells. Lymphocyte subsets were determined by flow cytometry (DakoCytomation CyanADP) after staining single-, double-, or quadruple-staining separate mononuclear cell aliquots from each rat with one of four panels of MAbs. Panel 1 (*Total Leukocytes*) contained PE-Cy5-conjugated mouse anti-rat CD45 (OX-1 clone; 1:200; BD-Pharmingen) which is reactive against all isoforms of the CD45 leukocyte common antigen found on all cells of hematopoetic origin except erythrocytes (Sunderland et al., 1979). Panel 2 (*B-cells and Granulocytes*) contained R-PE-conjugated mouse anti-rat CD45RA (OX-33 clone; 1:800; BD-Pharmingen) which reacts against the highest molecular weight form of CD45R (CD45RABC) specific for B-cells (Woollet et al., 1985) and FITC-conjugated mouse anti-rat HIS48 (1:100; Santa Cruz Biotechnology) which reacts against an antigen found on all granulocytes (Janeway et al., 1997). Panel 3 (*NK-cell*) contained R-PE-conjugated mouse anti-rat CD161 (10/78 clone; 1:800; BD-Pharmingen) which reacts with NKR-P1A, a homodimer expressed on all natural killer cells and a small proportion of T-cells (Kraus et al., 1996), and APC-conjugated mouse anti-rat CD3 (clone 1F4; 1:100; BD-Pharmingen) which is a T-cell receptor cell-surface antigen found on all thymocytes, peripheral T lymphocytes, and dendritic T cells (Elbe et al., 1994; Tanaka et al., 1989). Panel 4 (*T-cell*) contained the APC-conjugated CD3 antibody (1:100) as used in Panel 3; FITC-conjugated mouse anti-rat CD4 (W3/25 clone; 1:100; Santa Cruz Biotechnology) which reacts to the CD4 receptor on T cells and monocytes (White et al., 1978); PerCP-conjugated mouse anti-rat CD8a (clone OX8; 1:200; BD-Pharmingen) which reacts to the α-chain of the CD8 differentiation antigen found on most thymocytes and most T suppressor/cytotoxic cells (Barclay, 1981; Brideau et al., 1980; Mason et al., 1983; Torres-Nagel et al., 1992); and R-PE-conjugated mouse anti-rat CD-25 (OX39 clone; 1:200; BD-Pharmingen) which reacts to the α-chain of the high-affinity IL-2 receptor on T lymphocytes (Paterson et al., 1987). For each animal, a negative-control (incubation with no MAb added) sample was included, and for each panel, single-stained controls (incubation with only one of the component MAbs) were included for use in compensation analyses.

All analyses were performed using FlowJo version 6.3 (Tree Star Inc., Palo Alto, CA) on a Macintosh G4 computer. Lymphocyte and neutrophil subpopulations were identified and gated by using forward- versus side-scatter characteristics. Only the cells falling in this gate were forwarded to dot plots for evaluating antibody-specific fluorescence. Comparison with single-stain and negative stain control samples allowed discriminating specific from non-specific staining cell surface markers. Cutoffs for negative control staining were always <5% (and typically <1%) of the extreme tail of the distribution of single-stain controls. All samples were analyzed using the same gates, which were set to include 100% of cells labeled with MAb against: CD45 (*Panel 1*), CD45RA or HIS48 (*Panel 2*), CD161 but not CD3 (*Panel 3*), and CD3 (*Panel 4*). The number of fluorescent cells was expressed as the percentage of total leukocytes, which when multiplied by leukocyte concentration (obtained directly from whole blood samples) allowed calculation of specific leukocyte subtype concentration for each animal. For each staining antibody, the mean fluorescence intensity (MFI) of the positive cells obtained from each sample was used to measure the expression of surface molecules. For each molecule studied, all samples were analyzed on the cytometer with the same conditions of voltage.

Experiment 2: Effects of photoperiod on ingestive and somatic responses to simulated infection—One week after blood samples were obtained (*week 11*), rats from Experiment 1 were weighed, individually housed, and returned to their respective photoperiod chambers (LD: n=16; SD: n=16). One week later (*week 12*) rats received an i.p. injection of

either 125 μg/kg of *E. coli* lipopolysaccharide (LPS; serotype 0127:B8; Sigma; n=8 per photoperiod) or 0.9% sterile physiological saline (Saline; n=8 per photoperiod) 30 minutes before the onset of darkness. LPS is the biologically-active fragment of endotoxin from gram-negative bacteria but is non-replicating. This dose and strain of LPS was used because it reliably induces physiological and behavioral symptoms of infection in Wistar rats (Bluthe et al., 2001; Dogan et al., 2000). During the 4 days prior to, and for 17 days after, injection treatments, body mass was determined, and the uneaten portion of the animal's food (including small crumbs on the cage floor) was weighed (± 0.1 g) each day between 16:00 and 17:00 h to determine daily food intake. Body mass measurements were continued for an additional 7 days after measurements of food intake were terminated. Because body masses differed significantly between LD and SD rats, body mass and food intake were expressed as a percentage change from baseline (day of injection) values.

In addition to group-level comparisons, for each individual animal a somatic and an ingestive 'recovery time' was calculated as the number of days required for body mass and food intake to return to values that were greater than or equal to the individual's baseline value, and remained at or above this value for two or more successive days. One rat failed to exhibit full recovery of body mass to its baseline value by the end of the experiment; for statistical purposes, this rat was assigned a body mass recovery duration equal to the last day of the sampling interval (*day 17*).

Experiment 3: Effects of photoperiod on emotional and social behavior following infection—One week after arrival in the laboratory (*week 0*), 32 male rats were transferred to either a LD (16L:8D; n=16) or a SD (8L:16D; n=16) photoperiod as described in *Experiment 1*. Rats remained pair-housed for the next 7 weeks; during this time animals were weighed at weekly intervals. Beginning on *week 7*, rats were individually-housed, and on *week 8*, rats were injected i.p. with LPS (n=8 per photoperiod) or Saline (n=8 per photoperiod) in a manner identical to that described in *Experiment 2*. Rats were returned to their home cages and photoperiod chambers following injections, and novel object behavioral testing began 2–3 h later.

Novel object test: Novel object exploration tests were carried out during the dark phase of the light-dark cycle under dim red illumination. This test measures the tendency of an individual to explore a novel object under a situation of minimally perceived threat, as a means to assess the relative strength of two competing drives— exploration versus safety. The reaction to a novel object was assessed during three separate intervals (2–3 h, 24–25 h, and 48–49 h) after injections. The test was conducted in the animal's home cage and began when a small cup was placed upside-down at the end of the cage opposite (along the long axis) to where the rat sat in its nest. To minimize habituation to the object, cups of different sizes and materials were used for each of the three testing sessions (small plastic: 150 ml, translucent; small paper: 120 ml; medium plastic: 265 ml, opaque red). Rats had never been exposed to cup-shaped objects; thus the stimuli held no natural significance and had never been associated with a reinforcer. Exploration of the novel object was quantified as time spent touching, sniffing, or biting the cup. Behavior tests were recorded using a digital video camera equipped with an infrared filter (Sony; Model DCR-TRV260). Video records were scored using the Etholog transcription program on a PC computer (Ottoni, 2000), by an observer who was unaware of the treatment conditions.

Social exploration test: Following the completion of the novel object tests, rats remained single-housed in LD (n=16) or SD (n=16) until *week 12*. Body mass and food intake were measured on a daily basis during this interval to ensure that rats had fully recovered from the injection treatments of *week 8* (see *Results*). On *week 12*, social components of the sickness behavior induced by LPS were assessed by measuring the duration of social exploration of a

male juvenile (21–35 day old) conspecific. This is a validated procedure for measuring the intensity of social withdrawal during the acute response to simulated bacterial infection (Bluthe et al., 1992; 1999). One day before injection treatments, baseline levels of social exploration were measured by presenting experimental animals in their home cage with a juvenile male for 4 min (Bluthe et al., 1999). The duration of time spent by the experimental rat engaged in social investigation of the juvenile (anogenital sniffing, grooming, and body sniffing) during this interval was recorded (Bluthe et al., 1992). The following night rats were injected with LPS or Saline (as described in Experiment 2) 1 h before the onset of darkness. Social exploration tests lasting 4 min each were conducted in the home cage of each experimental animal 2, 6, 24, and 48 h after injection treatments under dim red illumination. Interactions were recorded on video, and the time spent engaged in social exploration was determined by an observer who was unaware of the experimental treatment conditions. To control for individual differences in the propensity to explore conspecifics, and to permit comparisons of relative changes in exploration levels, the baseline (pre-treatment) social exploration score was used as an internal control for each rat. Behavioral results were calculated as a percentage of the baseline social exploration measurement and are reported as means ± SEM.

Experiment 4: Effects of photoperiod on LPS-stimulated proinflammatory cytokine production—One week after arrival in the laboratory (*week 0*), 18 male rats were transferred to either a LD (16L:8D; n=9) or a SD (8L:16D; n=9) photoperiod as described in *Experiment 1*. Rats remained pair-housed for the next 8 weeks. At two week intervals between weeks 0 and 8, rats were weighed and their reproductive condition was assessed by measuring the length and width of the left testis, the product of which provides an estimated testis volume (ETV) which is highly (R^2>0.9) correlated with testis mass (Gorman & Zucker, 1995) and provides a reliable, non-invasive indicator of hypothalamic-pituitary-gonadal axis activity (Heideman & Sylvester, 1997). On *week 8* rats were individually-housed, and on *week 9* rats were injected i.p. with LPS (125 μg/kg) or 0.9% sterile physiological saline 30 minutes before the onset of darkness, in a counterbalanced design, with successive injections separated by 8 days.

Blood collection: One day (24 h) prior to injection, a 'baseline' blood sample (1.0 ml) was obtained under light isoflurane anesthesia (4% mixture with medical O_2) from the retroorbital sinus using a heparinized Pasteur pipette. To minimize stress, blood collections were performed in a room separate from the animal housing chambers. Following the blood withdrawal procedure, rats were separated from the colony until all blood collections for the day were completed. Animal handling during blood collection was also kept to a minimum (<2 min), and prior to recovery from anesthesia, rats were administered 1.0 ml of sterile 0.9% saline for rehydration. Additional blood samples were obtained 2, 6 and 10 h after LPS and Saline injections in an identical manner. Following collection, blood samples remained on ice for 1 h and were centrifuged at 2500 × *g* for 30 min at 4°C. Plasma was stored at −80°C until assayed for IL-1β and corticosterone concentrations by enzyme-linked immunosorbent assays (ELISAs; see below).

Determination of Hormone and Cytokine Concentrations: Corticosterone was measured using an ELISA (Correlate-EIA Kit, Assay Designs) according to the manufacturer's instructions. The ELISA uses a polyclonal antibody to corticosterone to competitively bind plasma corticosterone and alkaline-phosphatase conjugated corticosterone. Briefly, plasma samples were thawed at room temperature, diluted 1:30 in assay buffer, and applied to a microplate. Following incubation and robotic washing (SkanWasher 400, Molecular Devices, Sunnyvale, CA), plates were read on a microplate reader at 405 nm, and values were determined by extrapolation from a standard curve using SoftMaxPro 5.0 software (Molecular Devices).

The corticosterone ELISA had a sensitivity of <27.0 pg/ml, an intra-assay CV of 7.7% and an inter-assay CV of 9.7%. All samples, standards, and replicates were assayed in duplicate.

IL-1β was measured by ELISA (Quantikine, Rat IL-1β Kit; R&D Systems) according to the manufacturer's instructions. Briefly, plasma samples were thawed and centrifuged at room temperature, and diluted 1:3 in assay buffer. Diluted samples and standards were added in duplicate to a microplate coated with an affinity-purified polyclonal antibody specific for rat IL-1β. After binding to the immobilized antibody, wells were washed, HRP-conjugated secondary antibodies were added, wells were washed again, and a colorimetric reaction was performed to permit visualization of a reaction product. Finally, the optical density of each microplate well was read at 450 nm using a microplate reader (Emax, Molecular Devices) with correction at 570 nm. Sample values were determined by extrapolation from a standard curve. The IL-1β ELISA has a sensitivity of <5.0 pg/ml, an intra-assay CV of 5.5%, and an inter-assay CV of 4.6%. All samples, standards, and replicates were assayed in duplicate.

In a subset of samples which corresponded to the peak of cytokine production (+2 h and +6 h post-LPS and saline treatment; as determined in the time-course assay described above; see *Results*), IL-6 and TNFα protein concentrations were determined using the Bioplex Protein Array system (Bio-Rad, Hercules, CA), according to methods described in de Jager et al. (2003). Briefly, plasma samples were thawed at 20°C and diluted four-fold in sample diluent buffer. Diluted samples were assayed for cytokine levels (IL-2, IL-6, TNFα, and IFNg) using a multiplex bead-based immunoassay kit (no. 171-K11070; Bio-Rad). Cytokine assays were performed exactly as described by the manufacturer's protocol.

Statistical analyses: In Experiment 1, mean body masses and leukocyte counts were compared between photoperiods using student's t-tests. In Experiment 2, differences in body mass and food intake, and the number of days required to recover baseline body mass and food intake values were compared using student's t-tests. In Experiment 3, within-subjects factorial ANOVAs were used to compare changes in novel object and social investigation over time using a 2 (LD, SD) × 2 (LPS, Saline) repeated-measures design. Post hoc planned comparisons were conducted using t-tests between means. In Experiment 4, changes in testis volumes were compared using repeated-measures ANOVA with photoperiod (LD, SD) as a between-subjects factor and time (weeks of photoperiod) as the repeated measure. Differences in plasma corticosterone concentrations were compared using t-tests; LPS- and saline-induced changes in cytokine production were compared using paired and unpaired t-tests. Differences were considered significant if $p \leq 0.05$.

Results

Experiment 1: Effects of photoperiod on circulating leukocytes

Initial (*week 0*) body weights were comparable between LD and SD rats ($t_{30}=0.31$; $p>0.05$). Rats housed in SD gained body mass significantly slower than rats housed in LD (Fig. 1). A significant difference in body mass was first evident by week 3 ($t_{30}=2.21$, $p<0.05$), and persisted through week 10. At the time of blood sampling (week 10), LD rats weighed 35 ±10 g (~8%) more than SD rats.

Exposure to SD did not significantly affect the overall concentration of leukocytes in the circulation ($p>0.05$) but significantly affected the concentration of several leukocyte subtypes (Table 1). Total numbers of CD3+ T cells were significantly higher in SD relative to LD rats ($t_{30}=2.07$, $p<0.05$), as were the number of CD8+ T cells ($t_{30}=2.22$, $p<0.05$). CD4+ T cell concentrations were comparable among LD and SD rats ($t_{30}=1.60$, $p>0.05$); however, relative to LD rats, SD rats had significantly higher concentrations of CD25+/CD4+ T cells ($t_{30}=2.07$,

$p<0.05$) and CD25+/CD8+ T cells ($t_{30}=1.95$, $p<0.05$). NK cell, B cell, and granulocyte concentrations were comparable among LD and SD groups ($p>0.05$, all comparisons).

Experiment 2: Effects of photoperiod on ingestive and somatic responses to simulated infection

On the day of injection treatments (*week 12*), baseline daily food intake (an average of food intake measurements from the 4 days immediately preceding injections) was significantly greater in LD relative to SD rats ($t_{30}=2.07$, $p<0.05$; Fig. 2A), and LD rats weighed more than SD rats ($t_{30}=2.28$, $p<0.05$; Fig. 2B). In order to permit comparisons between photoperiod groups, changes in food intake and body mass subsequent to LPS/Saline treatments were expressed as a percentage of baseline values. Neither daily food intake ($F_{1,14}=2.6$, $p>0.05$) nor body mass accretion ($F_{1,14}=4.3$, $p>0.05$) differed between Saline-injected LD and SD animals during the 17 days following injection treatment.

LPS injections transiently suppressed food intake in both LD and SD rats (Fig. 3). The magnitude of suppression of food intake was comparable between LD and SD rats (LD nadir: −51±5%; SD nadir: 47±3%; $p>0.05$). However, LPS-induced suppression of food intake endured significantly longer in LD relative to SD rats. Group-wise analyses indicated that food intake among LPS-injected rats in both photoperiods was significantly lower than that of their respective saline-treated controls for the first 3 days following treatment; however, mean food intake among LPS-treated rats in LD was also significantly lower than that of saline-injected rats on *day 6*, *day 9*, and *day 10* ($p<0.05$, all comparisons), whereas food intake in LPS-injected SD rats did not differ from that of controls at any time point after *day 3*. Idiographic analyses indicated that recovery of baseline ingestive behavior required an average of 11.5 (±1.1) days in LD rats whereas baseline food intake was restored in 7.4 (±0.7) days in SD rats ($p<0.05$).

LPS injections likewise suppressed body mass gain in LD and SD rats (Fig. 4). Suppression of body mass was evident to a comparable magnitude in LD and SD rats (LD nadir: −3.4+0.4%; SD nadir: −2.8+0.4%; $p>0.05$). Suppression of body mass endured significantly longer in LPS-treated rats housed in LD relative to those in SD. Body masses of LPS-injected rats in SD returned to values that did not differ from those of saline-injected rats by *day 15*, whereas body masses of LPS-treated rats housed in LD did not return to values comparable to those of saline-injected animals ($p<0.05$, all comparisons). Longitudinal analyses of individual animals indicated that body mass recovery required an average of 14.3 (±1.2) days in LD rats versus 10.6 (±1.1) days in SD rats ($p<0.05$).

Experiment 3: Effects of photoperiod on emotional and social behavior following simulated infection

The duration of novel object exploration differed significantly according to injection treatment ($F_{1,28}=10.6$, $p<0.05$) and time ($F_{2,56}=11.8$, $p<0.05$), but not photoperiod ($F_{1,28}=3.60$, $p>0.05$; Fig. 5). The treatment × time interaction was significant ($F_{2,56}=3.42$, $p<0.05$). Post-hoc comparisons of mean exploration times indicated that photoperiod had no effect on novel object exploration in saline-injected rats at any time following treatment; however, LPS treatments significantly inhibited novel object exploration in LD animals when tested 2 h ($t_{14}=4.53$, $p<0.05$ vs. LD-saline) and 24 h ($t_{14}=3.46$, $p<0.05$ vs. LD-saline) after LPS treatment. In contrast, the duration of novel object exploration exhibited by SD rats injected with LPS did not differ from that of saline-injected SD rats at any time following treatment ($p>0.05$, all comparisons).

Baseline measures of the duration of social investigation of a conspecific juvenile (obtained on the night before injection treatments) were comparable across the four photoperiod and injection treatment groups ($t_{14}<0.79$, $p>0.05$, all comparisons). Three LPS-injected rats (2 LD,

1 SD) failed to exhibit decreases in body mass or food intake during the three days following LPS treatments; based on these independent criteria, these three animals were excluded from all behavioral analyses. Following LPS and saline injections, changes in the amount of social investigation differed significantly as a function of injection treatment ($F_{1,25}$=4.35, p<0.05), and time ($F_{4,100}$=21.3, p<0.05; Fig. 6); the treatment × time interaction was also significant ($F_{4,100}$=6.84, p<0.05). LPS-injected rats in LD and SD exhibited less social exploration than did their saline-injected counterparts 2 h after treatment (LD: t_{12}=3.29, p<0.05; SD: t_{13}=2.83, p<0.05); however, 6 h after treatment, social exploration duration did not differ between LPS- and saline-treated rats in SD (t_{13}=1.83, p>0.05), whereas LD-LPS rats were significantly less exploratory than LD-saline rats (t_{12}=2.36, p<0.05). Moreover, the magnitude of suppression of social exploration was significantly greater in LD relative to SD rats 2 h after LPS treatment (t_{11}=2.80, p<0.05). Photoperiod had no effect on social exploration in saline-injected rats at any time following treatment (p>0.05, all comparisons).

Experiment 4: Effects of photoperiod on corticosterone and LPS-induced IL-1β production

Between weeks 0 and 8, testis volume increased significantly in rats housed under both LD and SD ($F_{2,32}$=21.3, p<0.05); however, the pattern of this change was comparable among LD and SD rats ($F_{2,32}$<0.1, p>0.05; Fig. 7A). At no time point in the experiment did mean testis volume differ between LD and SD rats (t_{16}<0.3, p>0.05, all comparisons).

Prior to injection treatments, resting (baseline) corticosterone concentrations were significantly higher in SD as compared to LD rats (t_{16}=2.4, p<0.05; Fig. 7B).

Pre-injection IL-1β concentrations were low and comparable among LD and SD rats (t_{16}=0.1, p>0.05; Fig. 8). In both LD and SD rats, LPS induced significant increases in plasma IL-1β relative to baseline values: IL-1β was increased 2 h (LD: t_8=2.1, p<0.05; SD: t8=2.2, p<0.05), 6 h (LD: t_8=3.4, p<0.05; SD: t_8=3.5, p<0.05), and 10 h (LD: t_8=4.6, p<0.05; SD: t_8=3.1, p<0.05; Fig. 8) after injection. At none of these time points did IL-1β concentrations differ significantly between LD and SD animals (t_{16}<1.1, p>0.05, all comparisons). At the peak of LPS-induced IL-1β production (6 h post-injection), both LD and SD rats injected with LPS exhibited significantly higher IL-1β concentrations relative to those injected with saline (LD: t_{15}=2.0, p<0.05; SD: t_{15}=2.2, p<0.05; data not shown). Following saline-injections, IL-6 and TNFα concentrations were low and comparable between LD and SD rats (t_3<1.3; p>0.05, all comparisons, data not shown). IL-6 concentrations showed a tendency towards being higher in long-day LPS-treated rats relative to saline-injected rats 2 h after treatments (t_{11}=1.45; p=0.08), but among LPS-injected rats did not differ between LD and SD groups at 2 h (t_{16}=0.67; p>0.05) or at 6 h (t_{16}=0.93; p>0.05; Fig. 9) after treatment. TNFα concentrations were significantly elevated in LPS- relative to saline-treated rats (t_{11}>2.17; ps<0.05) and were significantly higher in the circulation of LD rats relative to SD rats 2 h after LPS treatment (t_{16}=1.89; p<0.05).

Discussion

Exposure to short photoperiods enhanced several aspects of the immune system of male Wistar rats. In blood, the number of T-cells (CD3+ and CD3+/CD8+) was augmented in short compared to long days, as were CD8+/CD25+ and CD4+/CD25+ T lymphocytes. Short days also attenuated acute phase sickness behaviors following a simulated bacterial infection. All rats exhibited anorexia and cachexia in response to LPS, but the duration of these symptoms was abbreviated in short- as compared to long-day rats. LPS also induced neophobia and social withdrawal, but the magnitude and persistence of these emotional symptoms were attenuated in short days. Photoperiod did not affect IL-1β or IL-6 responses to LPS, however, TNFα concentrations were nearly two-fold higher in long-day relative to short-day rats after LPS treatment. In addition, corticosterone concentrations were significantly elevated in short-

relative to long-day rats. Together, these results show that, in reproductively non-photoperiodic laboratory rats, exposure to short days affects multiple aspects of immune function: increasing the numbers of leukocytes capable of immunosurveillance and inhibition of inflammatory responses, decreasing innate immune responses (cytokine production) to simulated infection, and ultimately attenuating behavioral responses to infection.

The numbers of several subtypes of T lymphocytes were greater in rats exposed to short days. This enhancing effect of short photoperiods on T cells resembles that observed in reproductively photoperiodic rodents (e.g., Siberian hamsters; Bilbo et al., 2002a). In contrast to hamsters, the overall number of leukocytes in the blood did not differ in response to photoperiod, suggesting that increases in T-cell subtypes reflect a redistribution of investment in select cell phenotypes, rather than an omnibus effect on total leukocyte numbers. This redistribution raises the possibility that T-cell dependent, cell-mediated immune responses (e.g., memory T-cell dependent skin inflammatory responses; Dhabhar and McEwen, 1996) may likewise be enhanced in rats under short days, as is the case in Siberian hamsters (Bilbo et al., 2002a).

Behavioral symptoms of infection were attenuated in rats exposed to short days. Although LPS-induced anorexia was comparable in magnitude across photoperiods, it was markedly protracted in long-day rats, which required 3–4 additional days of recovery to exhibit ingestive behavior typical of baseline values. The net effect of this extended anorexic interval was an enduring decrement in body mass. Whereas short-day LPS-treated rats weighed as much as saline-injected controls within 15 days after treatment, long-day rats remained significantly lighter than controls for the entire post-injection measurement interval.

Short days also attenuated non-ingestive sickness behaviors. LPS inhibited novel object investigation for ≥ 24 h long-day rats, whereas this behavior was unaffected in short-day rats. In addition, both the magnitude and duration of LPS-induced inhibition of social investigation were greater in long-day as compared to short-day rats. The primary mediators of LPS-induced sickness behaviors are IL-1β, IL-6 and TNFα, which act both in the periphery and in the CNS to regulate ingestive behavior and emotional tone (Kent et al., 1992; Ek et al., 1998; Quan et al., 1998; Konsman et al., 2000; Dantzer et al., 2001). The present results suggest that functionally-significant aspects of this immune-neural pathway may be altered following adaptation to short photoperiods. These data are reminiscent of the attenuation of somatic, emotional, and ingestive symptoms of simulated infection in Siberian hamsters following adaptation to short days (Bilbo et al., 2002b).

Short photoperiod may attenuate rat sickness behaviors in part through changes in LPS-elicited proinflammatory cytokine production. Multiple cytokines participate in the genesis of sickness behavior, acting individually and in synergy (Dantzer, 1999; Dantzer et al., 2001). Although peripheral IL-1β and IL-6 responses to LPS did not differ between LD and SD rats, plasma TNFα concentrations were approximately two-fold higher in LD relative to SD rats 2 h after LPS treatment, matching the time course of the onset of exacerbated sickness behaviors in LD rats. Systemic administration of TNFα decreases social exploration in rats and mice (Bluthe et al., 1992; Bluthe et al., 1994) and is also sufficient to suppress feeding behavior under various paradigms (Kent et al., 1996; Plata-Salaman, 1999). TNFα, acting through the TNFR1 and TNFR2 receptor subtypes (Simen et al., 2006), is an important mediator of changes in ingestive behavior and behavioral depression that contribute to pathophysiology of LPS-induced sickness behavior (Dantzer et al., 2001). Thus rats, in common with hamsters (Prendergast et al., 2003a), exhibit reductions in TNFα responses to LPS under short days. Unlike hamsters (Bilbo et al., 2002b), however, rat IL-1β and IL-6 responses appear impervious to day length.

Baseline plasma corticosterone was significantly higher in short-day relative to long-day rats, an effect similar to the reported in Siberian hamsters (Bilbo et al., 2002a). It was beyond the scope of the present study to determine whether short-day increases in corticosterone are maintained throughout the circadian cycle; however, in hamsters short-day increases in cortisol are evident at several phases of the circadian cycle (including shortly before lights-off, as described here) and amplified (SD≫LD) following LPS treatments (Bilbo et al., 2003). Endogenous glucocorticoids have potent inhibitory effects on sickness behaviors (Goujon, et al., 1997), attenuating proinflammatory cytokine production in the periphery (Kapcala et al., 1995; Pezeshki et al., 1996; Goujon et al., 1996), and suppressing expression of proinflammatory cytokines in the CNS (Wisse et al., 2004). In rats, exogenous glucocorticoids also inhibit behavioral and thermoregulatory responses to LPS and IL-1β (McClellan et al., 1994; Morrow et al., 1996; Propes & Johnson, 1997). Thus, in addition to inhibiting cytokine production, short days may suppress the expression of sickness responses via a parallel effect on the HPA axis.

Increases in T lymphocytes under short days are also noteworthy in light of the short-day attenuation of sickness behaviors reported here. T cells expressing CD4 and CD25 were significantly upregulated in short days. Corticosterone treatment markedly enhances proliferation of CD4+CD25+ T lymphocytes *in vitro* (Wiegers et al., 2000), thus increased cell numbers in short day rats are consistent with short-day upregulation of HPA axis activity. CD4 +CD25+ T cells are commonly known as regulatory T cells (Tregs; Ziegler, 2006). Tregs can suppress proliferation of naive and memory T cells and inhibit production and release of proinflammatory cytokines through several mechanisms (Piccirillo & Thornton, 2004; Fontenot et al., 2005; Ziegler, 2006): Tregs suppress innate immune responses, inhibiting inflammation in part through production of IL-10 and TGFβ (Uhlig et al., 2006; Maloy et al., 2007). Treg cells also inhibit proinflammatory cytokine (IL-1β, IL-6, TNFα) responses to LPS (Murphy et al., 2005). It is possible that photoperiodic differences in Treg numbers participate in the attenuation of sickness behaviors through their suppressive effect on LPS-stimulated TNFα release. Further study is necessary to determine the extent to which short-day increases in T cell numbers participate in the photoperiodic regulation of cytokine and behavioral responses to LPS.

The adaptive significance of seasonal changes in immunity—principally the enhancement of peripheral leukocyte numbers and the suppression of sickness responses— under winter conditions— has been argued to lie in a redistribution of energy towards physiological systems responsible for host defense at a time of year when reproduction is contraindicated (Demas and Nelson, 1996; Nelson, 2004; Nelson and Demas, 1996). The photoperiodic attenuation of some energetically-costly acute phase symptoms, particularly anorexia, may also reflect an adaptive response by winter animals, given the relatively lower temperatures and reduced availability of food during winter. Behavioral suppression (whether directed towards novel objects or conspecifics) is also an energy-conserving response to infection: lethargy reduces heat loss and facilitates the maintenance of fever (Hart, 1988). The observation that short days either abolish (novel object) or attenuate (social investigation) LPS-induced behavioral depression in rats raises the possibility that some energetically-costly aspects of the acute response to infection may not be suppressed by exposure to winter cues. Given that the ancestral stock of laboratory rats, Norway rats, continue to forage throughout the winter, and typically live in communal social groups in which thermoregulation is facilitated by huddling (Calhoun, 1963), the short-day amelioration of LPS-induced behavioral depression may allow rats to resume food-motivated exploratory behavior sooner after an infection, and may also facilitate tolerance of the inevitable conspecific social interactions that are required for communal huddling.

Although reproductive photoperiodic responses have been reported in several inbred and outbred laboratory rats (Francisco et al., 2004; Heideman and Sylvester, 1997; Leadem, 1988; Lorincz et al., 2001; Shoemaker and Heideman, 2002); this is not the case for Wistar rats (Fig. 7; Vanecek and Illnerova, 1982; Wallen et al., 1987). Thus, this is the first report to demonstrate that non-reproductively photoperiodic rats exhibit alterations in multiple aspects of the immune system in response to changes in photoperiod. Immunological responsiveness to changes in photoperiod evidently do not depend on concurrent reproductive responses, suggesting that reproductive and immune responses to photoperiod are not necessarily linked in small mammals.

The mechanisms by which rats engage photoperiodic changes in the immune system are not fully known, but may involve seasonal changes in pineal melatonin production and glucocorticoid secretion. Nocturnal melatonin secretion reliably tracks seasonal changes in photoperiod (Illnerova and Vanecek, 1980), and pinealectomy abolishes photoperiodic gonadal responses in reproductively photoresponsive rodents (including select rat strains: [Heideman and Sylvester, 1997; Prendergast et al., 2002a]). Pineal melatonin may figure prominently in the transduction of day length information into the immune system of rats, as is the case in hamsters (Wen et al., 2007). Substrates in the immune system and in the CNS that participate in the generation of sickness behaviors may respond directly or indirectly to changes in melatonin secretion (Bilbo & Nelson, 2002; Hotchkiss & Nelson, 2002). In addition, photoperiodic changes in corticosterone secretion may also constitute a post-pineal mechanism relevant to seasonal changes in the immune system. The control of the HPA axis by photoperiod and melatonin in reproductively non-photoperiodic mammals clearly warrants further investigation.

Wistar rats are one of the most commonly-used models for investigations of basic immunological processes. The extent to which formal and physiological aspects of photoperiodic time measurement in immunologically-photoperiodic rats and reproductively-photoperiodic rodents are similar is not presently known. However, the neuroendocrine mechanisms responsible for photoperiodic time measurement are exquisitely sensitive to changes in illumination at night (Hoffmann et al., 1981; Whaling et al., 1993; Gorman and Elliott, 2004) and prior ‘photoperiod history’ effects (Weaver et al., 1987; Prendergast et al., 2000). Standard rat husbandry protocols may inadvertently engage photoperiodic responses with undesired consequences.

In summary, photoperiod regulates leukocyte numbers, cytokine production, and sickness behaviors in laboratory rats. Decreases in proinflammatory cytokine production and increases in corticosterone production under short days may provide mechanisms by which day length alters the immune response to infection. The present data indicate that reproductive and immune responses to photoperiod are not obligately linked, and thus are consistent with the hypothesis that, following the perception of a change in photoperiod by the CNS, parallel but independent mechanisms may regulate photoperiodic responses in the reproductive and immune systems.

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References

Ansar Ahmed S, Penhale WJ, Talal N. Sex hormones, immune responses, and autoimmune diseases. Mechanisms of sex hormone action. Am J Pathol 1985;121:531–551. [PubMed: 3907369]

Barclay AN. The localization of populations of lymphocytes defined by monoclonal antibodies in rat lymphoid tissues. Immunology 1981;42:593–600. [PubMed: 7016746]

Bhat GK, Hamm ML, Igietseme JU, Mann DR. Does leptin mediate the effect of photoperiod on immune function in mice? Biol Reprod 2003;69:30–36. [PubMed: 12606383]

Bilbo SD, Dhabhar FS, Viswanathan K, Saul A, Yellon SM, Nelson RJ. Short day lengths augment stress-induced leukocyte trafficking and stress-induced enhancement of skin immune function. Proc Natl Acad Sci USA 2002a;99:4067–4072. [PubMed: 11904451]

Bilbo SD, Drazen DL, Quan N, He L, Nelson RJ. Short day lengths attenuate the symptoms of infection in Siberian hamsters. Proc Biol Sci 2002b;269:447–454. [PubMed: 11886635]

Bilbo SD, Nelson RJ. Sex steroid hormones enhance immune function in male and female Siberian hamsters. Am J Physiol 2001;280:R207–R213.

Bilbo SD, Nelson RJ. Melatonin regulates energy balance and attenuates fever in Siberian hamsters. Endocrinology 2002;143:2527–2533. [PubMed: 12072384]

Bilbo SD, Quan N, Prendergast BJ, Bowers SL, Nelson RJ. Photoperiod alters the time course of brain cyclooxygenase-2 expression in Siberian hamsters. J Neuroendocrinol 2003;15:958–964. [PubMed: 12969240]

Bluthe RM, Bristow A, Lestage J, Imbs C, Dantzer R. Central injection of interleukin-13 potentiates LPS-induced sickness behavior in rats. Neuroreport 2001;12:3979–3983. [PubMed: 11742223]

Bluthe RM, Castanon N, Pousset F, Bristow A, Ball C, Lestage J, Michaud B, Kelley KW, Dantzer R. Central injection of IL-10 antagonizes the behavioural effects of lipopolysaccharide in rats. Psychoneuroendocrinology 1999;24:301–311. [PubMed: 10101735]

Bluthe RM, Dantzer R, Kelley KW. Effects of interleukin-1 receptor antagonist on the behavioral effects of lipopolysaccharide in rat. Brain Res 1992;573:318–320. [PubMed: 1387028]

Bluthe RM, Pawlowski M, Suarez S, Parnet P, Pittman Q, Kelley KW, Dantzer R. Synergy between tumor necrosis factor alpha and interleukin-1 in the induction of sickness behavior in mice. Psychoneuroendocrinology 1994;19:197–207. [PubMed: 8190839]

Brideau RJ, Carter PB, McMaster WR, Mason DW, Williams AF. Two subsets of rat T lymphocytes defined with monoclonal antibodies. Eur J Immunol 1980;10:609–615. [PubMed: 6967416]

Bronson, FH. Mammalian Reproductive Biology. University of Chicago Press; Chicago: 1989.

Calhoun, JB. The ecology and sociology of the Norway rat. U.S. Government Printing Office; Bethesda: 1963.

Dantzer R. Cytokine-induced sickness behavior: mechanisms and implications. Ann N,Y, Acad, Sci 1999;933:222–234. [PubMed: 12000023]

Dantzer R. Cytokine-induced sickness behavior: where do we stand? Brain Behav Immun 2001;15:7–24. [PubMed: 11259077]

de Jager W, Velthuis H, Prakken BJ, Kuis W, Rijkers GT. Simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells. Clin Diagn Lab Immunol 2003;10:133–139. [PubMed: 12522051]

Demas GE, Klein SL, Nelson RJ. Reproductive and immune responses to photoperiod and melatonin are linked in *Peromyscus* subspecies. J Comp Physiol A 1996;179:819–825. [PubMed: 8956499]

Demas GE, Nelson RJ. Photoperiod and temperature interact to affect immune parameters in adult male deer mice (*Peromyscus maniculatus*). J Biol Rhythms 1996;11:94–102. [PubMed: 8744237]

Demas GE, Nelson RJ. Photoperiod, ambient temperature, and food availability interact to affect reproductive and immune function in adult male deer mice (*Peromyscus maniculatus*). J Biol Rhythms 1998a;13:253–262. [PubMed: 9615289]

Demas GE, Nelson RJ. Short-day enhancement of immune function is independent of steroid hormones in deer mice (*Peromyscus maniculatus*). J Comp Physiol B 1998b;168:419–426. [PubMed: 9747522]

Desjardins C, Bronson FH, Blank JL. Genetic selection for reproductive photoresponsiveness in deer mice. Nature 1986;322:172–173. [PubMed: 3724859]

Dhabhar FS, McEwen BS. Stress-induced enhancement of antigen-specific cell- mediated immunity. J Immunol 1996;156:2608–2615. [PubMed: 8786326]

Dogan MD, Ataoglu H, Akarsu ES. Effects of different serotypes of *Escherichia coli* lipopolysaccharides on body temperature in rats. Life Sci 2000;67:2319–2329. [PubMed: 11065179]

Drazen DL, Kriegsfeld LJ, Schneider JE, Nelson RJ. Leptin, but not immune function, is linked to reproductive responsiveness to photoperiod. Am J Physiol 2000;278:R1401–R1407.

Elbe A, Kilgus O, Hunig T, Stingl G. T-cell receptor diversity in dendritic epidermal T cells in the rat. J Invest Dermatol 1994;102:74–79. [PubMed: 7904617]

Ek M, Kurosawa M, Lundeberg T, Ericsson A. Activation of vagal afferents after intravenous injection of interleukin-1beta: role of endogenous prostaglandins. J Neurosci 1998;18:9471–9479. [PubMed: 9801384]

Fontenot JD, Rudensky AY. A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. Nat Immunol 2005;6:331–337. [PubMed: 15785758]

Francisco NR, Raymond CM, Heideman PD. Short photoperiod inhibition of growth in body mass and reproduction in ACI, BUF, and PVG inbred rats. Reproduction 2004;128:857–862. [PubMed: 15579603]

Gatien ML, Hotchkiss AK, Neigh GN, Dhabhar FS, Nelson RJ. Immune and stress responses in C57BL/6 and C3H/HeN mouse strains following photoperiod manipulation. Neuro Endocrinol Lett 2004;25:267–272. [PubMed: 15361815]

Goldman BD. Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. J Biol Rhythms 2001;16:283–301. [PubMed: 11506375]

Gorman MR, Elliott JA. Dim nocturnal illumination alters coupling of circadian pacemakers in Siberian hamsters, *Phodopus sungorus*. J Comp Physiol A 2004;190:631–639.

Gorman MR, Zucker I. Testicular regression and recrudescence without subsequent photorefractoriness in Siberian hamsters. Am J Physiol 1995;269:R800–R806. [PubMed: 7485596]

Goujon E, Laye S, Parnet P, Dantzer R. Regulation of cytokine gene expression in the central nervous system by glucocorticoids: mechanisms and functional consequences. Psychoneuroendocrinology 1997;22:S75–80. [PubMed: 9264151]

Goujon E, Parnet P, Laye S, Combe C, Dantzer R. Adrenalectomy enhances pro-inflammatory cytokines gene expression, in the spleen, pituitary and brain of mice in response to lipopolysaccharide. Brain Res Mol Brain Res 1996;36:53–62. [PubMed: 9011765]

Hart B. Biological basis of the behavior of sick animals. Neurosci Biobehav Rev 1988;12:123–137. [PubMed: 3050629]

Heideman PD, Sylvester CJ. Reproductive photoresponsiveness in unmanipulated male Fischer 344 laboratory rats. Biol Reprod 1997;57:134–138. [PubMed: 9209091]

Hoffmann K, Illnerova H, Vanecek J. Effect of photoperiod and of one minute light at night-time on the pineal rhythm on N-acetyltransferase activity in the Djungarian hamster *Phodopus sungorus*. Biol Reprod 1981;24:551–556. [PubMed: 7195287]

Horton TH. Cross-fostering of voles demonstrates in utero effect of photoperiod. Biol Reprod 1985;33:934–939. [PubMed: 3910122]

Hotchkiss AK, Nelson RJ. Melatonin and immune function: hype or hypothesis? Crit Rev Immunol 2002;22:351–371. [PubMed: 12803316]

Illnerova H, Vanecek J. Pineal rhythm in N-acetyltransferase activity in rats under different artificial photoperiods and in natural daylight in the course of a year. Neuroendocrinology 1980;31:321–326. [PubMed: 7442935]

Illnerova H, Vanecek J, Krecek J, Wetterberg L, Saaf J. Effect of one minute exposure to light at night on rat pineal serotonin N-acetyltransferase and melatonin. J Neurochem 1979;32:673–675. [PubMed: 762577]

Janeway, JA.; Travers, P.; Hunt, S.; Walport, M. Immunobiology: the immune system in health and disease. Garland Publishing; New York: 1997.

Kapcala LP, Chautard T, Eskay RL. The protective role of the hypothalamic-pituitary adrenal axis against lethality produced by immune, infectious, and inflammatory stress. Ann N Y Acad Sci 1995;771:419–437. [PubMed: 8597419]

Kent S, Bluthe RM, Dantzer R, Hardwick AJ, Kelley KW, Rothwell NJ, Vannice JL. Different receptor mechanisms mediate the pyrogenic and behavioral effects of interleukin 1. Proc Natl Acad Sci 89:9117–9120.

Kent S, Bret-Dibat JL, Kelley KW, Dantzer R. Mechanisms of sickness-induced decreases in food-motivated behavior. Neurosci Biobehav Rev 1996;20:171–175. [PubMed: 8622824]

Kinsey SG, Prendergast BJ, Nelson RJ. Photoperiod and stress affect wound healing in Siberian hamsters. Physiol Behav 2003;78:205–211. [PubMed: 12576117]

Klein SL. The effects of hormones on sex differences in infection: from genes to behavior. Neurosci Biobehav Rev 2000;24:627–638. [PubMed: 10940438]

Konsman JP, Luheshi GN, Bluthe RM, Dantzer R. The vagus nerve mediates behavioural depression, but not fever, in response to peripheral immune signals; a functional anatomical analysis. Eur J Neurosci 2000;12:4434–4446. [PubMed: 11122354]

Kraus E, Lambracht D, Wonigeit K, Hunig T. Negative regulation of rat natural killer cell activity by major histocompatibility complex class I recognition. Eur J Immunol 1996;26:2582–2586. [PubMed: 8921942]

Leadem CA. Photoperiodic sensitivity of prepubertal female Fisher 344 rats. J Pineal Res 1988;5:63–70. [PubMed: 3367261]

Lorincz AM, Shoemaker MB, Heideman PD. Genetic variation in photoperiodism among naturally photoperiodic rat strains. Am J Physiol 2001;281:R1817–R1824.

Loscher W, Mevissen M, Haussler B. Seasonal influence on 7,12-dimethylbenz[a]-anthracene-induced mammary carcinogenesis in Sprague-Dawley rats under controlled laboratory conditions. Pharmacol Toxicol 1997;81:265–270. [PubMed: 9444667]

Mahmoud I, Salman SS, al-Khateeb A. Continuous darkness and continuous light induce structural changes in the rat thymus. J Anat 1994;185:143–149. [PubMed: 7559109]

Maloy KJ, Salaun L, Cahill R, Dougan G, Saunders NJ, Powrie F. CD4+CD25+ T(R) cells suppress innate immune pathology through cytokine-dependent mechanisms. J Exp Med 2003;197:111–119. [PubMed: 12515818]

Mason DW, Arthur RP, Dallman MJ, Green JR, Spickett GP, Thomas ML. Functions of rat T-lymphocyte subsets isolated by means of monoclonal antibodies. Immunol Rev 1983;74:57–82. [PubMed: 6226588]

McClellan JL, Klir JJ, Morrow LE, Kluger MJ. Central effects of glucocorticoid receptor antagonist RU-38486 on lipopolysaccharide and stress-induced fever. Am J Physiol 1994;267:R705–R711. [PubMed: 8092314]

Morrow LE, McClellan JL, Klir JJ, Kluger MJ. The CNS site of glucocorticoid negative feedback during LPS- and psychological stress-induced fevers. Am J Physiol 1996;271:R732–R737. [PubMed: 8853398]

Murphy TJ, Choileain N, Zang Y, Mannick JA, Lederer JA. CD4+CD25+ regulatory T cells control innate immune reactivity after injury. J Immunol 2005;174:2957–2963. [PubMed: 15728508]

Nelson RJ. Seasonal immune function and sickness responses. Trends Immunol 2004;25:187–192. [PubMed: 15039045]

Nelson RJ, Demas GE. Seasonal changes in immune function. Q Rev Biol 1996;71:511–548. [PubMed: 8987173]

Nelson, RJ.; Demas, GE.; Klein, SL.; Kriegsfeld, LJ. Seasonal Patterns of Stress, Immune Function, and Disease. Cambridge University Press; New York: 2002.

Nelson RJ, Moffatt CA, Goldman BD. Reproductive and nonreproductive responsiveness to photoperiod in laboratory rats. J Pineal Res 1994;17:123–131. [PubMed: 7897584]

Nelson RJ, Zucker I. Photoperiodic control of reproduction in olfactory-bulbectomized rats. Neuroendocrinology 1981;32:266–271. [PubMed: 7242854]

Ottoni EB. EthoLog 2.2: a tool for the transcription and timing of behavior observation sessions. Behav Res Methods Instrum Comput 2000;32:446–449. [PubMed: 11029818]

Paterson DJ, Jefferies WA, Green JR, Brandon MR, Corthesy P, Puklavec M, Williams AF. Antigens of activated rat T lymphocytes including a molecule of 50,000 Mr detected only on CD4 positive T blasts. Mol Immunol 1987;24:1281–1290. [PubMed: 2828930]

Pelegri C, Rodriguez-Palmero M, Morante MP, Comas J, Castell M, Franch A. Comparison of four lymphocyte isolation methods applied to rodent T cell subpopulations and B cells. J Immunol Methods 1995;187:265–271. [PubMed: 7499886]

Pezeshki G, Pohl T, Schobitz B. Corticosterone controls interleukin-1 beta expression and sickness behavior in the rat. J Neuroendocrinol 1996;8:129–135. [PubMed: 8868260]

Piccirillo CA, Thornton AM. Cornerstone of peripheral tolerance: naturally occurring CD4+CD25+ regulatory T cells. Trends Immunol 2004;25:374–380. [PubMed: 15207505]

Plata-Salaman CR. The 1998 Curt P. Richter Award. Brain mechanisms in cytokine- induced anorexia. Psychoneuroendocrinology 1998;24:25–41. [PubMed: 10098218]

Prendergast BJ, Bilbo SD, Dhabhar FS, Nelson RJ. Effects of photoperiod history on immune responses to intermediate day lengths in Siberian hamsters (*Phodopus sungorus*). J Neuroimmunol 2004;149:31–39. [PubMed: 15020062]

Prendergast BJ, Bilbo SD, Nelson RJ. Short day lengths enhance skin immune responses in gonadectomised Siberian hamsters. J Neuroendocrinol 2005;17:18–21. [PubMed: 15720471]

Prendergast BJ, Gorman MR, Zucker I. Establishment and persistence of photoperiodic memory in hamsters. Proc Natl Acad Sci USA 2000;97:5586–5591. [PubMed: 10792054]

Prendergast BJ, Hotchkiss AK, Bilbo SD, Kinsey SG, Nelson RJ. Photoperiodic adjustments in immune function protect Siberian hamsters from lethal endotoxemia. J Biol Rhythms 2003a;18:51–62. [PubMed: 12568244]

Prendergast BJ, Hotchkiss AK, Nelson RJ. Photoperiodic regulation of circulating leukocytes in juvenile Siberian hamsters: mediation by melatonin and testosterone. J Biol Rhythms 2003b;18:473–480. [PubMed: 14667148]

Prendergast, BJ.; Nelson, RJ.; Zucker, I. Mammalian seasonal rhythms: Behavior and neuroendocrine substrates. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. Hormones, Brain and Behavior. Academic Press; San Diego: 2002a. p. 93-156.

Prendergast BJ, Wynne-Edwards KE, Yellon SM, Nelson RJ. Photorefractoriness of immune function in male Siberian hamsters (*Phodopus sungorus*). J Neuroendocrinol 2002;14:318–329. [PubMed: 11963829]

Propes MJ, Johnson RW. Role of corticosterone in the behavioral effects of central interleukin-1 beta. Physiol Behav 1997;61:7–13. [PubMed: 8976527]

Quan N, Whiteside M, Herkenham M. Time course and localization patterns of interleukin-1beta messenger RNA expression in brain and pituitary after peripheral administration of lipopolysaccharide. Neuroscience 1998;83:281–293. [PubMed: 9466417]

Schneider JE. Energy balance and reproduction. Physiol Behav 2004;81:289–317. [PubMed: 15159173]

Shoemaker MB, Heideman PD. Reduced body mass, food intake, and testis size in response to short photoperiod in adult F344 rats. BMC Physiol 2002;2:11. [PubMed: 12135532]

Simen BB, Duman CH, Simen AA, Duman RS. TNFalpha signaling in depression and anxiety: behavioral consequences of individual receptor targeting. Biol Psychiatry 2006;59:775–785. [PubMed: 16458261]

Sunderland CA, McMaster WR, Williams AF. Purification with monoclonal antibody of a predominant leukocyte-common antigen and glycoprotein from rat thymocytes. Eur J Immunol 1979;9:155–159. [PubMed: 374095]

Tanaka T, Masuko T, Yagita H, Tamura T, Hashimoto Y. Characterization of a CD3-like rat T cell surface antigen recognized by a monoclonal antibody. J Immunol 1989;142:2791–2795. [PubMed: 2467940]

Torres-Nagel N, Kraus E, Brown MH, Tiefenthaler G, Mitnacht R, Williams AF, Hunig T. Differential thymus dependence of rat CD8 isoform expression. Eur J Immunol 1992;22:2841–2848. [PubMed: 1358623]

Uhlig HH, Coombes J, Mottet C, Izcue A, Thompson C, Fanger A, Tannapfel A, Fontenot JD, Ramsdell F, Powrie F. Characterization of Foxp3+CD4+CD25+ and IL-10-secreting CD4+CD25+ T cells during cure of colitis. J Immunol 2006;177:5852–5860. [PubMed: 17056509]

Vanecek J, Illnerova H. Effect of photoperiod on the growth of reproductive organs and on pineal N-acetyltransferase rhythm in male rats treated neonatally with testosterone propionate. Biol Reprod 1982;27:517–522. [PubMed: 6890385]

Wallen EP, DeRosch MA, Thebert A, Losee-Olson S, Turek FW. Photoperiodic response in the male laboratory rat. Biol Reprod 1987;37:22–27. [PubMed: 3651546]

Wallen EP, Turek FW. Photoperiodicity in the male albino laboratory rat. Nature 1981;289:402–404. [PubMed: 6780913]

Weaver DR, Keohan JT, Reppert SM. Definition of a prenatal sensitive period for maternal-fetal communication of day length. Am J Physiol 1987;253:E701–E704. [PubMed: 3425712]

Wen JC, Dhabhar FS, Prendergast BJ. Pineal-dependent and -independent effects of photoperiod on immune function in Siberian hamsters (*Phodopus sungorus*). Horm Behav 2007;51:31–39. [PubMed: 17022983]

Whaling CS, Kelly KK, Finley CM, Spears N, Licht P, Zucker I. Sustained hormonal responses of Siberian hamsters (*Phodopus sungorus*) to a single longer day at weaning. Biol Reprod 1993;49:555–560. [PubMed: 8399850]

White RA, Mason DW, Williams AF, Galfre G, Milstein C. T-lymphocyte heterogeneity in the rat: separation of functional subpopulations using a monoclonal antibody. J Exp Med 1978;148:664–673. [PubMed: 29936]

Wiegers GJ, Stec IE, Klinkert WE, Reul JM. Glucocorticoids regulate TCR-induced elevation of CD4: functional implications. J Immunol 2000;164:6213–6220. [PubMed: 10843673]

Wisse BE, Ogimoto K, Morton GJ, Wilkinson CW, Frayo RS, Cummings DE, Schwartz MW. Physiological regulation of hypothalamic IL-1beta gene expression by leptin and glucocorticoids: implications for energy homeostasis. Am J Physiol Endocrinol Metab 2004;287:E1107–E1113. [PubMed: 15304373]

Woollett GR, Barclay AN, Puklavec M, Williams AF. Molecular and antigenic heterogeneity of the rat leukocyte-common antigen from thymocytes and T and B lymphocytes. Eur J Immunol 1985;15:168–173. [PubMed: 2578966]

Yellon SM, Fagoaga OR, Nehlsen-Cannarella SL. Influence of photoperiod on immune cell functions in the male Siberian hamster. Am J Physiol 1999;276:R97–R102. [PubMed: 9887182]

Yellon SM, Tran LT. Photoperiod, reproduction, and immunity in select strains of inbred mice. J Biol Rhythms 2002;17:65–75. [PubMed: 11837950]

Ziegler SF. FOXP3: of mice and men. Annu Rev Immunol 2006;24:209–226. [PubMed: 16551248]

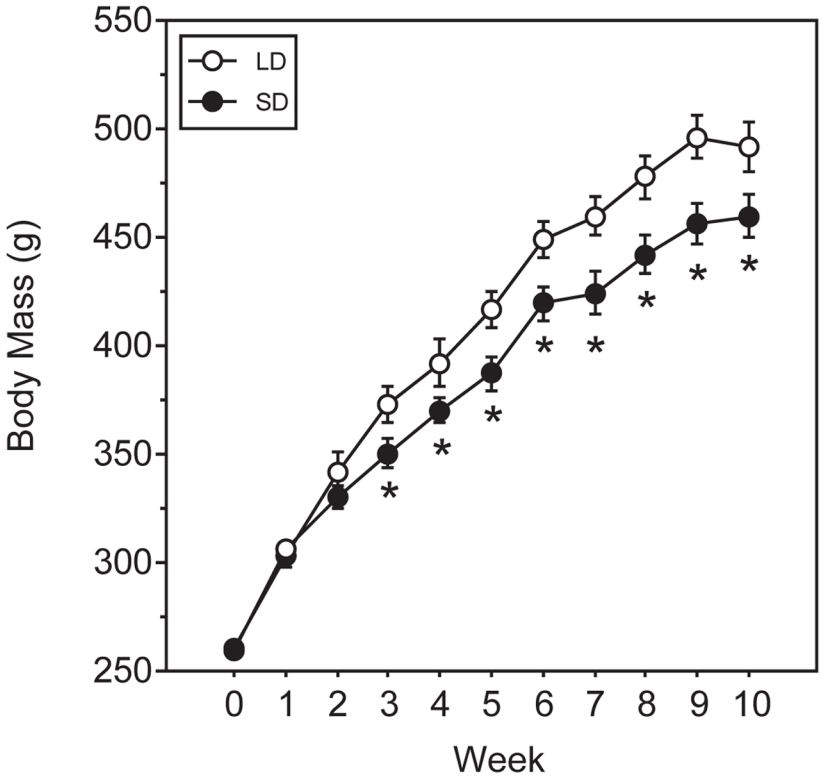

Figure 1.
Mean (±SEM) body mass of adult male Wistar rats housed 2 per cage and maintained on lighting regimens (photoperiods) of either 16L:8D (LD) or 8L:16D (SD). Prior to the inception of LD and SD treatments, rats were housed in a 12L:12D photoperiod. * $p<0.05$ vs. LD value.

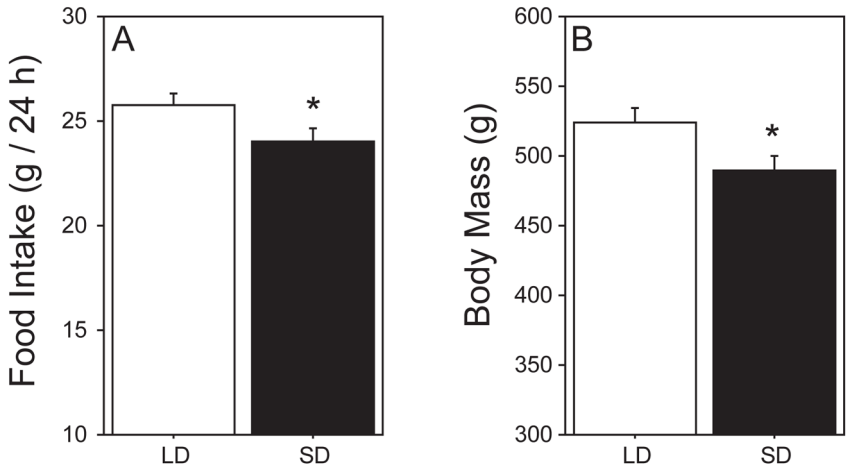

Figure 2.

Mean (±+SEM) body mass and food intake of adult male Wistar rats housed in 16L:8D (LD) or 8L:16D (SD) for 12 weeks. Rats were housed 2 per cage from week 0 until week 8, and singly housed thereafter. * $p<0.05$ vs. LD value.

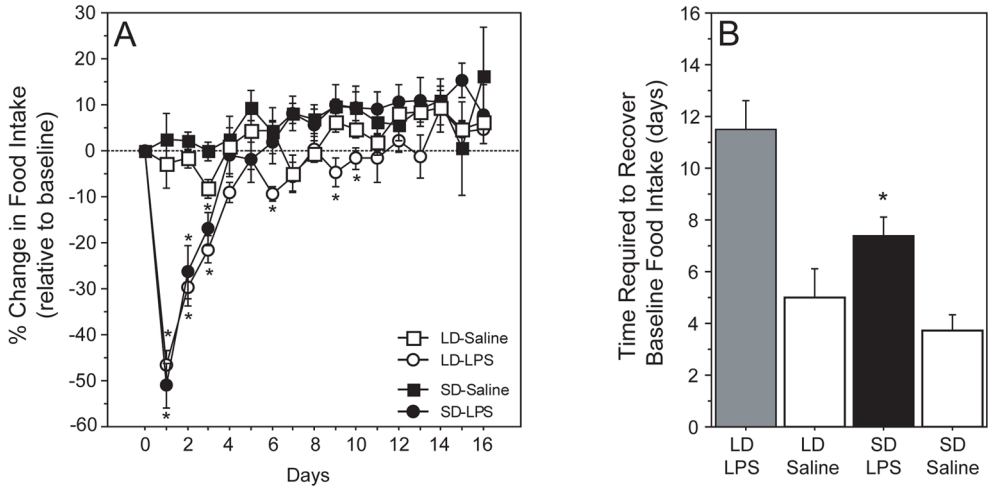

Figure 3.

(A) Mean (±SEM) percent change in daily food intake from baseline in adult male Wistar rats following injection with bacterial lipopolysaccharide (125 μg/kg, i.p.; LPS) or sterile saline (Saline) on day 0. Prior to injection treatments, rats were housed in long-day (16 h light/day; LD) or short-day (8 h light/day; SD) photoperiods for 12 weeks. *$p<0.05$ vs. Saline value. (B) Mean (+SEM) number of days to recover baseline food intake. *$p<0.05$ vs. LD value.

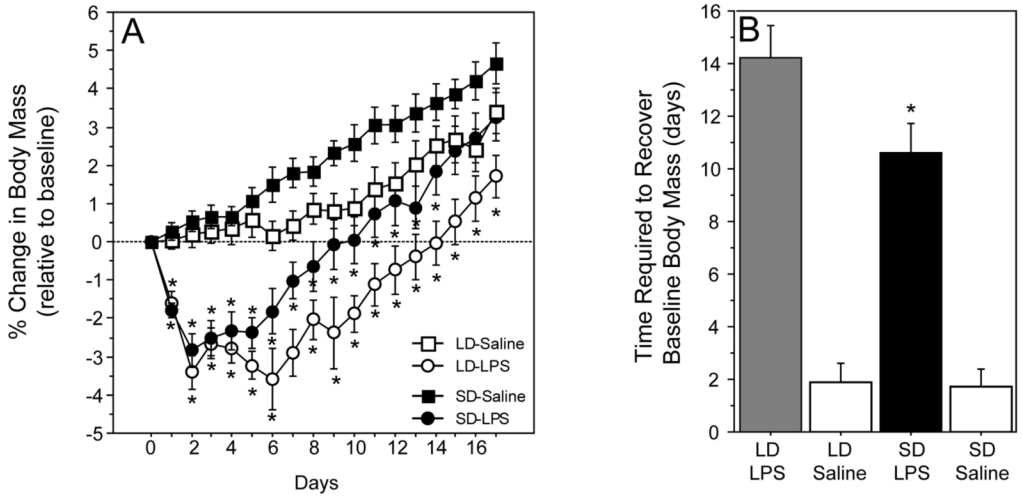

Figure 4.

(A) Mean (±SEM) percent change in body mass from baseline in adult male Wistar rats following injection with bacterial lipopolysaccharide (125 μg/kg, i.p.; LPS) or sterile saline (Saline) on day 0. Prior to injection treatments, rats were housed in long-day (16 h light/day; LD) or short-day (8 h light/day; SD) photoperiods for 12 weeks. *$p<0.05$ vs. Saline value. (B) Mean (+SEM) number of days to recover baseline body mass. *$p<0.05$ vs. LD value.

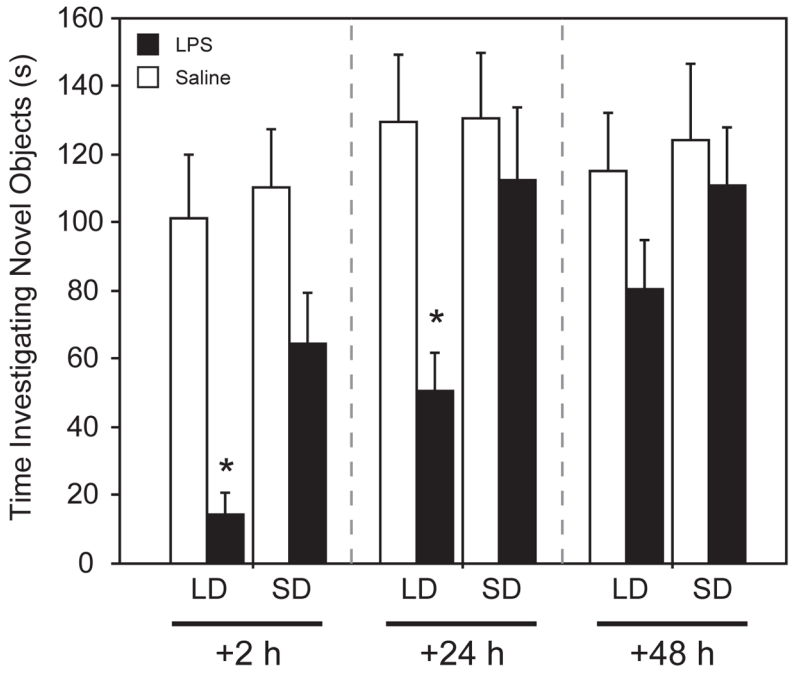

Figure 5.

Mean (+SEM) time spent engaged in exploration of a novel object by adult male Wistar rats following injection with bacterial lipopolysaccharide (125 μg/kg, i.p.; LPS) or sterile saline (Saline). Novel object tests were conducted in the home cage 2, 24, and 48 h after LPS and Saline treatments. Prior to injections, rats were housed in long-day (16 h light/day; LD) or short-day (8 h light/day; SD) photoperiods for 8 weeks. *$p<0.05$ vs. Saline-treated group in the same photoperiod.

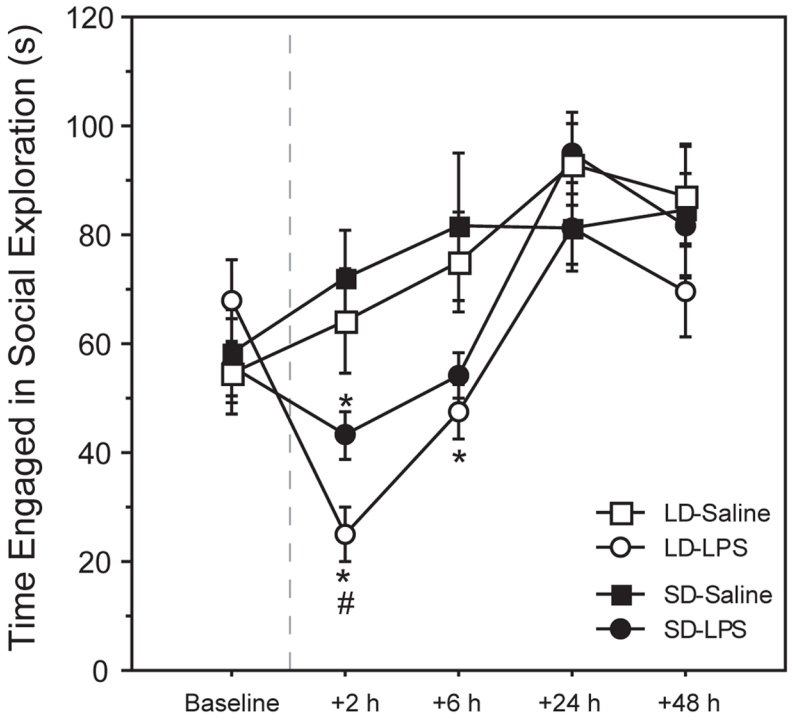

Figure 6.

Mean (±SEM) time spent engaged in social exploration of a novel juvenile conspecific by adult male Wistar rats following injection with bacterial lipopolysaccharide (125 μg/kg, i.p.; LPS) or sterile saline (Sal). Social exploration tests were conducted in the home cage 2, 6, 24, and 48 h after LPS and Sal treatments. Prior to injections, rats were housed in long-day (16 h light/day; LD) or short-day (8 h light/day; SD) photoperiods for 12 weeks. *$p<0.05$ vs. Saline-treated group in the same photoperiod. #$p<0.05$ vs. LPS-treated SD value.

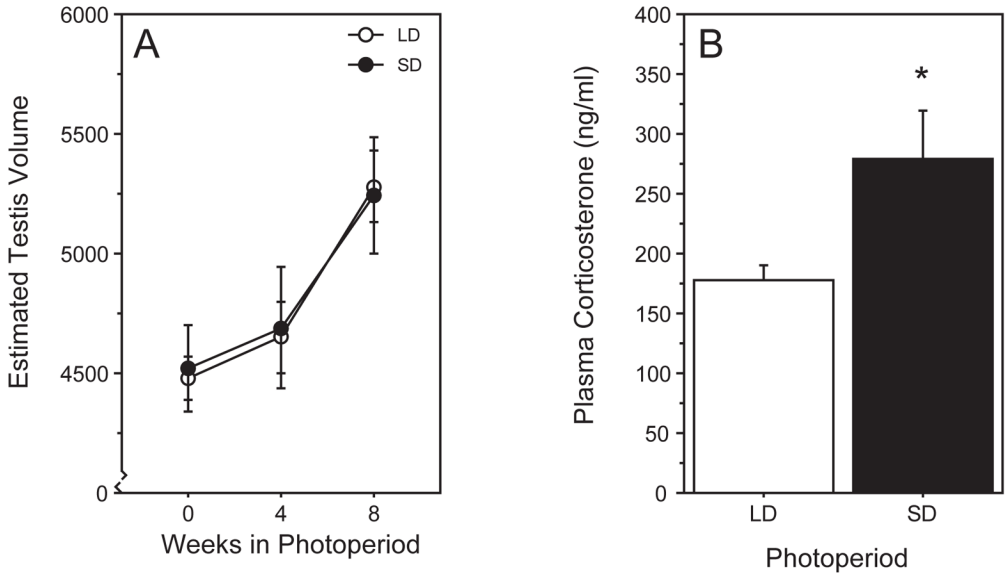

Figure 7.

(A) Mean (±SEM) estimated testis volume of adult male Wistar rats transferred to long-day (16 h light/day; LD) or short-day (8 h light/day; SD) photoperiods beginning on *week 0*. Prior to the inception of LD and SD treatments, rats were housed in a 12L:12D photoperiod. Note discontinuity in ordinate axis. (B) Mean (+SEM) plasma corticosterone concentrations in LD and SD housed rats as determined on week 9. *$p<0.05$ vs. LD value.

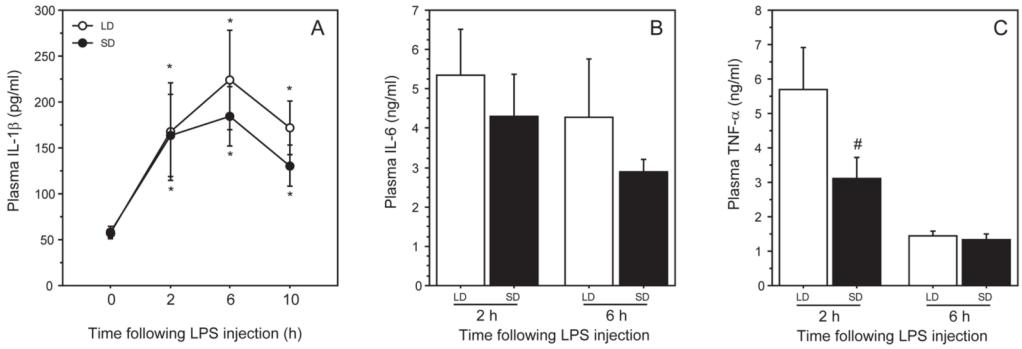

Figure 8.

Mean (±SEM) LPS-induced (A) plasma IL-1β, (B) plasma IL-6, and (C) plasma TNFα concentrations of adult male Wistar rats following exposure to long day (16 h light/day; LD) or short-day (8 h light/day; SD) photoperiods for 9–10 weeks. Rats were injected with 125 μg/kg E. coli LPS 30 minutes before the onset of darkness. *$p<0.05$ relative to baseline (0 h) value within photoperiod. #$p<0.05$ vs. LD value at the same time point.

Table 1

Mean (±SEM) leukocyte concentrations (cells × 10^3/μl whole blood) of male Wistar rats housed in long (16L:8D) or short (8L:16D) photoperiods for 8 weeks.

Cell phenotype	Long days	Short days
Total leukocytes	11.69 (±0.6)	13.24 (±0.6)
Lymphocytes	9.27 (±0.5)	10.45 (±0.5)
CD3+ T-cells	4.14 (±0.3)	4.98 (±0.3)*
CD8+ T-cells	1.13 (±0.1)	1.49 (±0.1)*
CD4+ T-cells	2.81 (±0.2)	3.24 (±0.2)
CD3+/CD8+/CD25+ T-cells	0.09 (±0.01)	0.11 (±0.01)*
CD3+/CD4+/CD25+ T-cells	0.24 (±0.02)	0.32 (±0.03)*
CD45RA+ B-cells	2.95 (±0.2)	3.20 (±0.3)
CD161+ NK cells	0.38 (±.03)	0.41 (±.04)
HIS48+ Granulocytes	3.04 (±0.2)	3.45 (±0.3)

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