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Influence of the olfactory bulbs on blood leukocytes and behavioral responses to infection in Siberian hamsters

Brian J. Prendergast^{a,b,*}, Jerome Galang^a, Leslie M. Kay^{a,b}, and Leah M. Pyter^a

^aDepartment of Psychology, Institute for Mind and Biology, The University of Chicago, Chicago, IL 60637, USA

^bCommittee on Neurobiology, The University of Chicago, Chicago, IL 60637, USA

Abstract

Surgical removal of the olfactory bulb alters several aspects of immunological activity. This study investigated the role of the olfactory bulbs in the control of behavioral responses to simulated infection, and the environmental modulation of sickness behaviors by changes in day length. Adult male Siberian hamsters (*Phodopus sungorus*) were subjected to bilateral olfactory bulbectomy (OBx) or a sham surgical procedure, and were then exposed to long (15 h light/day; LD) or short (9 h light/day; SD) photoperiods for 8–12 weeks, after which circulating leukocytes and behavioral responses (anorexia, anhedonia, cachexia) to simulated gram-negative bacterial infections (i.p. lipopolysaccharide [LPS] treatment; 0.625 mg/kg) were quantified. OBx treatment altered the effects of photoperiod on immune function in a trait-specific manner. LPS-induced anorexia was exacerbated in SD-OBx hamsters; LPS-induced anhedonia was exacerbated in LD-OBx hamsters; and photoperiodic differences in circulating leukocytes and LPS-induced cachexia were eliminated by OBx. Plasma cortisol concentrations did not differ between LD and SD hamsters, irrespective of olfactory bulb integrity. The data indicate that photoperiod affects immune function via OB-dependent and -independent mechanisms, and that changes in cortisol production are not required for photoperiodic changes in sickness behaviors to manifest.

Keywords

Immune function; Olfactory bulbectomy; Photoperiodism; Inflammation; Sickness behavior; Seasonality; Cortisol

1. Introduction

In response to the passing of the seasons, organisms engage changes in morphology, physiology, and behavior (Bronson, 1989). Among the more striking of these seasonal transitions are those that occur in the immune system, and in behavioral consequences of immune challenge, in small mammals. Rats (Prendergast et al., 2007), mice (Demas and Nelson, 1998), and hamsters (Drazen et al., 2000) exhibit marked seasonal changes in

multiple aspects of immune function, including the number and distribution of leukocyte phenotypes (Bilbo et al., 2003; Yellon et al., 1999), antibody production (Drazen et al., 2000), and inflammatory responses (Bilbo et al., 2003; Bilbo et al., 2002a). Changes in day length (photoperiod) serve as the principal environmental cue for inducing seasonal changes in immune function (Prendergast et al., 2009).

In Siberian hamsters, short photoperiods (<12 h light/day) trigger changes in behavioral components of the immune response. The acute-phase response to bacterial infection—a suite of changes in physiology and behavior, including proinflammatory cytokine production, fever, anorexia, behavioral depression, social withdrawal (Hart, 1988)—is markedly greater in magnitude and in duration among hamsters housed in long, summer-like photoperiods, relative to those that have been exposed to short, winter-like photoperiods (Bilbo et al., 2002b). Hamsters housed in long days also typically have lower plasma concentrations of immunomodulatory glucocorticoids (Bilbo et al., 2002a) and are substantially more behaviorally-responsive to proinflammatory cytokines (Wen and Prendergast, 2007). Because food is scarce and ambient temperatures are relatively lower during winter, decreases in energetically-expensive sickness behaviors in short days may optimize energy use; these behavioral changes have been argued to facilitate overwinter survival (Nelson, 2004).

Neural substrates that participate in the reorganization of sickness behaviors following changes in photoperiod are not fully cataloged. Pineal melatonin production is required for hamsters to discriminate long from short photoperiods for the purposes of engaging seasonal phenotypic changes in the acute phase response (Wen et al., 2007), and this action is mediated at the hypothalamic suprachiasmatic nucleus (Freeman et al., 2007). Cortical and non-hypothalamic limbic structures have yet to be implicated in the seasonal control of sickness behaviors.

The role of the olfactory bulb (OB) in the regulation of the immune system *in vivo* has received limited study. In mice, bilateral olfactory bulbectomy (OBx) increases the numbers of helper T cells in blood (Komori et al., 2002) and decreases constitutive production of proinflammatory cytokines (Novoselova et al., 2004). In rats, OBx increases tonic synthesis of proinflammatory cytokines in limbic (hippocampus, hypothalamus) and prefrontal regions (Myint et al., 2007), and in the circulation (Connor et al., 2000); however, relative to neurologically-intact animals, bulbectomized rats exhibit lower levels of peripheral cytokine production in response to a simulated infection (accomplished via peripheral treatment with lipopolysaccharide [LPS], the immunogenic component of gram-negative bacteria; Breivik et al., 2006; Connor et al., 2000). OBx-induced changes in cytokine production in response to LPS may be mediated directly or may occur secondary to OBx-induced increases in corticosterone production (Cairncross et al., 1977; Goujon et al., 1996; Song and Leonard, 1995). Proinflammatory cytokine production is necessary and sufficient for behavioral symptoms of infection to manifest (Dantzer, 2004), nevertheless, the consequences of OBx-induced changes in cytokine production on behavioral responses to infection have not been investigated. *In vivo* data suggest that, in rats, the magnitude of LPS-induced sickness behaviors would be diminished following OBx.

Therefore, this experiment investigated the neural bases of behavioral responses to infection by determining the contributions of the OB to the seasonal reorganization of sickness behaviors in Siberian hamsters. If, in common with rats and mice, OBx attenuates LPS-induced inflammatory responses, then an intact OB may be required to generate maximal behavioral responses to LPS challenge. If so, then OBx should attenuate the effects of long days on LPS-induced sickness behavior. Because sickness behaviors are already attenuated in hamsters housed in short days, OBx may have relatively little effect on sickness behaviors in short days.

2. Results

2.1. Histological assessment of bulbectomies

Necropsies revealed 6 complete OB lesions (no tissue remaining in the ‘olfactory chamber’) and 10 incomplete OB lesions (4 LD, 6 SD). In the latter animals, the amount of tissue remaining ranged from 1.3 to 7.2 mg (mean $\pm$ sd: 4.1 ± 2.1 mg). For all OBx hamsters, this exceeded the ‘completeness’ threshold of 80% removal as described by Bittman et al. (1989).

2.2. Effects of bulbectomy on humoral responses to photoperiod

2.2.1. Blood leukocytes—Circulating leukocyte numbers were significantly affected by photoperiod ($F_{1,36} = 16.3$, $P < 0.0005$), but not by surgical condition ($F < 0.1$, $P > 0.8$; photoperiod $\times$ surgery: $F = 1.7$, $P > 0.2$; Fig. 1A). Exposure to SD caused a significant increase in leukocyte concentrations among intact hamsters ($P < 0.0005$ vs. LD-intact), whereas the effect of SD treatments in SD-OBx hamsters was not significant ($P > 0.1$ vs. LD-OBx).

2.2.2. Plasma cortisol—Neither photoperiod ($F = 1.3$, $P > 0.2$) nor surgical condition ($F = < 0.1$, $P > 0.9$) affected plasma cortisol concentrations (Fig. 1B).

2.3. Effects of bulbectomy on behavioral and somatic responses to photoperiod

2.3.1. Home-cage activity—OBx induced a significant increase in general locomotor activity ($F_{1,36} = 5.4$, $P < 0.05$), which did not vary as a function of photoperiod ($F_{1,36} = 3.0$, $P > 0.05$; Fig. 2).

2.3.2. Food intake—OBx increased food intake ($F_{1,36} = 6.5$, $P < 0.05$), but the main effect of photoperiod ($F = 0.1$, $P > 0.9$) and the photoperiod surgical $\times$ condition interaction ($F = 2.0$, $P > 0.1$) were not significant (Fig. 3A). This main effect of OBx was largely driven by a significant increase in food intake in SD-OBx hamsters relative to intact SD hamsters ($P < 0.01$).

2.3.3. Saccharin intake—Overall, OBx increased saccharin intake ($F_{1,36} = 5.3$, $P < 0.05$). Neither photoperiod ($F_{1,36} = 1.5$, $P > 0.2$) nor the photoperiod $\times$ surgical condition interaction was significant ($F_{1,36} = 1.4$, $P > 0.2$) for this measure (Fig. 3B). The main effect of OBx was primarily a result of an increase in saccharin consumption in LD-OBx hamsters ($P < 0.05$ vs. LD-intact and SD-intact hamsters).

2.3.4. Body mass—Bulbectomy had no main effect on body mass ($F_{1,36} = 0.34$, $P > 0.5$), but short days tended to decrease body mass ($F_{1,36} = 3.83$, $P < 0.06$; Fig. 3C). Surgery and photoperiod did not interact to affect body mass ($F_{1,36} = 1.53$, $P > 0.2$). LD-sham hamsters weighed significantly more than SD-sham hamsters ($P < 0.05$).

2.4. Effects of bulbectomy on behavioral and somatic responses to simulated infection

2.4.1. LPS-induced anorexia—There was a significant three-way interaction among photoperiod $\times$ surgical condition $\times$ injection on food intake ($F_{3,216} = 5.99$, $P < 0.001$; Fig. 4). Among intact hamsters, changes in food intake were significantly affected by injection ($F_{1,44} = 65.8$, $P < 0.0001$) and photoperiod ($F_{1,44} = 8.9$, $P < 0.005$), and an interaction between injection and photoperiod ($F_{1,44} = 8.0$, $P < 0.01$; Fig. 4A). Food intake was decreased in hamsters injected with LPS compared with those treated with saline; this decrease persisted for 4 days in LD hamsters, and for 2 days in SD hamsters ($P < 0.05$, all comparisons). LPS-induced decreases in food intake were significantly greater in LD relative to SD hamsters on day +2, day +3, and day +4 ($P < 0.005$, all comparisons).

In OBx hamsters, LPS treatments likewise inhibited food intake ($F_{1,44} = 20.1$, $P = 0.0001$), but this effect did not vary as a function of photoperiod ($F_{1,28} = 0.2$, $P > 0.6$; photoperiod $\times$ injection: $F_{1,28} = 0.6$, $P > 0.4$; Fig. 4B). LPS-induced anorexia endured for 4 days in LD-OBx hamsters, and for 3 days in SD-OBx hamsters ($P < 0.05$, all comparisons). The magnitude of LPS-induced anorexia was statistically indistinguishable among LD-OBx and SD-OBx hamsters at all time points following injection ($P > 0.05$, all comparisons).

There was no main effect of bulbectomy on food intake following injections ($F_{1,66} = 0.6$, $P > 0.4$), and there was no significant interaction between photoperiod and bulbectomy on anorexic responses to LPS ($F_{1,66} = 0.6$, $P > 0.4$).

2.4.2. LPS-induced anhedonia—Photoperiod, surgical condition, and injection treatments interacted to affect saccharin consumption ($F_{1,72} = 8.7$, $P < 0.005$; Fig. 5). Among intact hamsters, LPS treatments inhibited saccharin intake ($F_{1,44} = 9.4$, $P < 0.005$; Fig. 5A). No main effect of photoperiod was evident ($F_{1,44} = 0.1$, $P > 0.7$), but LD and SD hamsters differed in the pattern of hedonic responsiveness to LPS: following LPS injection, saccharin intake was significantly suppressed on night 0 in LD-intact hamsters, whereas in SD-intact hamsters, LPS failed to significantly suppress saccharin intake on night 0 or on any other night, relative to saline-injected controls. The magnitude of LPS-induced anhedonia did not differ between LD and SD hamsters on any night ($P > 0.2$, all comparisons).

LPS treatments also inhibited saccharin intake among OBx hamsters ($F_{1,28} = 7.7$, $P < 0.01$), and this effect varied as a function of photoperiod ($F_{1,28} = 4.5$, $P < 0.05$; photoperiod $\times$ injection: $F_{1,28} = 9.1$, $P = 0.005$; Fig. 5B). LPS suppressed saccharin intake in LD hamsters on night 0, night 1, and night 2 ($P < 0.005$, all comparisons), whereas saccharin intake was not suppressed in SD hamsters on any night after LPS treatment ($P > 0.1$, all comparisons). The magnitude of LPS-induced anhedonia was comparable between LD-OBx and SD-OBx hamsters on all nights ($P > 0.2$, all comparisons).

Overall, bulbectomy significantly affected the pattern of saccharin intake following injection treatments, ($F_{1,66} = 9.2$, $P < 0.005$) and did so differently in LD relative to SD ($F_{1,66} = 4.6$, $P < 0.05$).

2.4.3. LPS-induced cachexia—There was a significant three-way interaction among photoperiod $\times$ surgical condition $\times$ injection on changes in body mass ($F_{5,360} = 3.68$, $P < 0.005$; Fig. 6). In all groups LPS-treated hamsters exhibited significant decreases in body mass beginning on day +1 and continuing through day +5 ($P < 0.05$, all comparisons). Among intact hamsters, there was a significant interaction between photoperiod and injection treatment on the pattern of body mass loss ($F_{5,220} = 6.97$, $P < 0.0001$; Fig. 6A). LPS-induced body mass loss was significantly greater among LD relative to SD hamsters on days +4 and +5.

Among OBx hamsters, LPS treatments caused significant decreases in body mass ($F_{5,140} = 3.26$, $P < 0.01$), but these changes were unaffected by photoperiod ($F_{5,140} = 0.90$, $P > 0.4$; Fig. 6B). Body mass loss among LD-OBx and SD-OBx hamsters did not differ in magnitude at any time point following treatment ($P > 0.4$, all comparisons).

There was no main effect of bulbectomy on body mass following injection treatments ($F_{1,66} < 0.1$, $P > 0.9$), and there was no significant interaction between photoperiod and bulbectomy on somatic responses to LPS ($F_{1,66} = 0.1$, $P > 0.7$).

2.5. Analyses of effect sizes

ω^2_A values were obtained for the effects of photoperiod on leukocyte counts, and on the patterns of change in food intake, saccharin intake, and body mass following LPS treatment (Table 1). In bulb-intact hamsters, large effects of photoperiod were detected for leukocyte counts and for LPS-induced changes in food intake and body mass; moderate effects were observed on changes in saccharin intake after LPS treatment. Among OBx hamsters, moderate-strength effects of photoperiod were observed for leukocyte counts, but effects of photoperiod on sickness responses to LPS were all 0.

3. Discussion

The neural substrates that contribute to photoperiodic regulation of mammalian physiology have traditionally focused on thalamic and hypothalamic nuclei controlling reproductive physiology (Badura and Goldman, 1992; Glass and Lynch, 1981), and on pituitary tissues that appear to control prolactin secretion (Prendergast et al., 2009). Although in Syrian hamsters the OB figures prominently in the regulation of the reproductive response to photoperiod (Pieper et al., 1989). Brain regions involved in the regulation of steady-state immune parameters and behavioral responses to infection have likewise focused on hypothalamic and limbic areas (Dantzer, 2004), but, prior to the present report, the role of the OB in the *in vivo* generation of integrated immune responses has gone largely uninvestigated. In Siberian hamsters, OBx was without effect on blood leukocyte counts and circulating cortisol, and resulted in modest changes in the behavioral responses to simulated infection and the photoperiodic regulation thereof. As observed in several other rodent models (e.g., Roche et al., 2008), and confirmed in the present experiment, OBx caused

photoperiod-independent increases in locomotor activity, characteristic of psychomotor agitation (Connor et al., 2000). The results are consistent with several lines of evidence suggesting that the olfactory bulbs influence select aspects of immune function (Breivik et al., 2006; Connor et al., 2000; Komori et al., 2002; Myint et al., 2007; Song and Leonard, 1995). In general, the data are compatible with the hypothesis that photoperiodic changes in several aspects of immune function and immunomodulatory glucocorticoid secretion are accomplished via olfactory bulb-dependent and -independent mechanisms.

Consistent with several prior reports (e.g., Bilbo et al., 2002b; Freeman et al., 2007; Wen et al., 2007), exposure to short days caused increases in circulating leukocytes and attenuated anorexic, anhedonic, and somatic responses to LPS treatments. The effects of long and short photoperiods on blood leukocyte numbers were attenuated slightly in OBx hamsters, yielding intermediate values that did not differ between photoperiods. Anorexic responses to LPS were unaffected by OBx in LD hamsters (4 days of significant LPS-induced anorexia) but slightly exacerbated by OBx in SD hamsters (3 days of LPS-induced anorexia among SD-OBx hamsters vs. 2 days among SD-Sham hamsters). The effect of photoperiod on body mass loss following LPS was essentially abolished by OBx, yielding body mass values among LD-OBx and SD-OBx hamsters that were intermediate between those of sham-operated LD and SD hamsters. Lastly, in LD-OBx hamsters an enduring anhedonia was evident following LPS treatment; however, this effect appeared to be driven by sharp increases in saccharin intake among saline-injected hamsters shortly after injection treatments, the reasons for which are not clear. Together, the data point to OBx inducing modest changes in the effects of photoperiod on immune function, which differ in direction and magnitude in a trait-specific manner. These outcomes were contrary to predictions based on the reported effects of OBx on cytokine production in rats and mice (Breivik et al., 2006; Connor et al., 2000). These differences may reflect species differences in the role of the OB on inflammatory responses, or may suggest that OBx-induced changes in cytokine production are insufficient in magnitude to culminate in measurable changes at the level of behavior.

Gonadal responses to photoperiod are capable of substantial immunomodulatory effects in this species (Prendergast et al., 2008a), but a recent report from our laboratory indicates that the Siberian hamster OB is entirely unnecessary for normal photoperiodic regulation of the reproductive system (Prendergast et al., 2008b). Entrainment of the circadian system to long and short days (and presumably, the downstream generation of photoperiod-specific nightly melatonin signals) is likewise comparable among OBx and intact hamsters, eliminating differences in entrainment as a potential mechanistic explanation for the modest effects of OBx observed. This is in marked contrast to the Syrian hamster, in which OBx alters entrainment and abolishes reproductive responses to short days (Bittman et al., 1989; Pieper et al., 1984), via elimination of tonic inhibitory projections from the OB to the hypothalamic GnRH neuron population (Pieper et al., 1989). Whether the diminished role of the OB in the photoperiodic control of the Siberian hamster immune system reflects simply another species difference in the role of the OB, or is more generally indicative of the role of the OB in neural-immune interactions remains to be determined.

Neither photoperiod nor OBx affected cortisol secretion in the present study. OBx is reported to have variable effects on the regulation of the HPA axis, ranging from substantial increases in basal and stress-induced glucocorticoid production (Cairncross et al., 1977; Cattarelli and Demael, 1986; Kelly et al., 1997; Marcilhac et al., 1999) to no effects (Montilla et al., 1984; Pistovcakova et al., 2008; Williams et al., 1992). Conflicting results have also been reported on the effects of long and short days on circulating cortisol concentrations in Siberian hamsters (Bilbo and Nelson, 2003; Weil et al., 2007; Yellon, 2007; Zysling and Demas, 2007). In light of the marked photoperiodic differences in sickness behaviors among sham-operated hamsters in the present study, the data suggest that photoperiodic changes in cortisol secretion are not necessary to mediate the effects of photoperiod on sickness behaviors, nor are any of the modest effects of OBx on immunity likely to be mediated by changes in cortisol. Rather, effects of photoperiod on immunity appear to be independent of the effects of photoperiod on the HPA axis.

There are numerous additional pathways by which OBx may alter immune function. OBx results in changes in concentrations or activity of serotonin, dopamine, noradrenaline, glutamate, acetylcholine, and GABA in a regionally-specific manner (Song and Leonard, 1995). Any of these neurotransmitter changes may have widespread effects on behavior or central production of cytokines (Dantzer, 2004). The neural mechanisms by which OBx affects immune function were beyond the scope of the present study.

Contrary to predictions based on effects of OBx on cytokine production in inbred rodent models, OBx did not attenuate sickness behaviors solely in long-day Siberian hamsters. Rather, effects of OBx were modest in magnitude, and were trait- and photoperiod-specific. Taken together, the data support only a minor role for the activity of the olfactory bulbs in the mediation of photoperiod-induced seasonal changes in immune function.

4. Experimental procedures

4.1. Animals and housing conditions

Male Siberian hamsters (*Phodopus sungorus*) were obtained from a breeding colony maintained at the University of Chicago. Hamster pups were weaned at 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 4–5 months 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*. All procedures conformed to the USDA Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Chicago.

4.2. Surgical procedures

Hamsters were subjected to surgical olfactory bulbectomy (OBx; $n = 25$) or a sham-OBx procedure ($n = 24$) under sodium pentobarbital anesthesia (Nembutal, 0.05 mg/g, i.p.; (Pieper et al., 1984). Hamsters were immobilized in a stereotaxic apparatus and a small (~2

mm) hole was drilled in the frontal bone near the caudal extent of the nasal bone. Bilateral bulbectomies were performed using a modified 200 μ l pipette tip. OBx was performed by bilateral aspiration of the olfactory bulbs from the anterior border of the olfactory bulbs to the frontal poles, without disturbing the superior sagittal sinus. This procedure removes all neural pathways from the olfactory bulbs to the brain, including the main olfactory bulb, the accessory olfactory bulb, and the nervus terminalis (Pieper et al., 1994).

The sham-OBx procedure entailed drilling of the skull and a comparable amount of blood loss, without insertion of the aspiration pipette. After surgery, hamsters received analgesia (Buprenex, 0.5 μ g/g, s.c.) twice per day for 2 successive days. A total of 16 OBx hamsters survived >2 weeks. At this time, hamsters were randomly assigned to long and short day photoperiods (sham: $n = 12$ /photoperiod; OBx: $n = 7$ LD, $n = 9$ SD).

4.3. Photoperiod treatments

Two weeks after surgery (= week 0), hamsters were transferred either into short days (SD; 9 h light/day, lights on at 0900 h CST) or remained in long days (LD; 15 h light/day). Hamsters were weighed (± 0.1 g) weekly, and estimated testis volumes (ETVs) were determined at two-week intervals under light isoflurane anesthesia. In this experiment, reproductive neuroendocrine responses to photoperiod (testis sizes, plasma testosterone and FSH concentrations) in OBx hamsters were comparable to those of sham-operated hamsters and have been reported elsewhere (Prendergast et al., 2008b).

4.4. Locomotor activity measures

Home cage activity data were collected using passive infrared motion detectors (Coral Plus, Visonic, Bloomfield, CT) positioned 22 cm above the cage floor. Motion detectors registered activity whenever 3 of 27 zones were crossed. Activity triggered closure of an electronic relay, which was recorded by a PC running ClockLab software (Actimetrics, Evanston, IL). The timing of activity was analyzed using ClockLab software according to methods described by Evans et al. (Evans et al., 2004). Briefly, a 24-h histogram was produced for each hamster by averaging activity counts in 5-min time bins over a 7–10 day window. For each histogram, activity onset was defined as the first 5-min bin after 1400 h with average counts exceeding the daily mean level; activity offset was defined as the last time point exceeding this threshold. The total amount of nocturnal activity was calculated as the mean number of activity counts occurring between activity onset and offset.

4.5. Immunological measures and behavioral responses to infection

4.5.1. Leukocyte counts—On week 8, blood samples (250 μ l) 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 Natleson collection tubes according to methods previously reported (Freeman et al., 2007). Following blood collection, hamsters were administered 0.5 ml sterile 0.9% saline s.c. for rehydration. 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. Animal handling during the blood collection was also kept to a minimum (<1 min). Whole blood samples were kept at room temperature for <3 h before whole blood

leukocyte counts were determined. Leukocyte counts from a 25 μ l aliquot of the whole blood sample were obtained by hemolysis with 3% acetic acid at a 1:20 dilution, and enumeration in duplicate on a hemacytometer at 400 $\times$ magnification. Although distinct leukocyte subtypes are not identifiable with this method, this procedure reliably identifies photoperiod- and stress-induced changes in total leukocyte number in this, and other, rodent species (Bilbo et al., 2002a; Dhabhar et al., 1995; Wen et al., 2007). In all prior studies of photoperiodic regulation of hamster leukocytopoiesis, total leukocyte counts have correlated positively with photoperiodic changes in specific leukocyte subtypes, including total lymphocytes, T-cells, and NK cells (Bilbo et al., 2002a; Prendergast et al., 2004; Wen et al., 2007). This measure therefore provides an omnibus indicator of treatment effects on the capacity for immunosurveillance in the blood (Freeman et al., 2007; Prendergast et al., 2003), obviating the need for large volume blood withdrawal that may affect subsequent behavioral tests.

4.5.2. Behavioral symptoms of infection—Beginning on week 10, anorexic and anhedonic sickness responses to LPS were evaluated according to established methods in our laboratory (Wen and Prendergast, 2007). Food intake and body mass measurements were obtained daily (at 1730 h, 30 min before the onset of darkness); consumption of a highly palatable 0.1% saccharin solution was recorded during the first 3 h of the dark phase (Baillie and Prendergast, 2008). After 3 days of baseline measurements, shortly before lights-off (1730–1800 h), hamsters were injected i.p. with either bacterial lipopolysaccharide (LPS, 625 μ g/kg; isolated from *E. coli* strain 026:B6, Lot 064K4077, Sigma, St. Louis, MO) or sterile 0.9% saline (saline; 0.1 ml), in a counter-balanced design, with successive injections separated by 7 days. Food intake, saccharin intake, and body mass measures were obtained for 5 successive days following injections.

4.6. Determination of hormone concentrations

Cortisol was measured in a single ELISA (Correlate-EIA; Assay Designs, Ann Arbor, MI, USA) that has been validated for this species (Demas et al., 2004) according to the manufacturer's instructions. The cortisol ELISA had a sensitivity of <57 pg/ml, an intra-assay CV of <10.5% and an inter-assay CV of <8.6%. Samples were measured in duplicate.

4.7. Histological verification of OB integrity

Histological examination of the olfactory bulbs was performed at necropsy. Mean OB mass was calculated for all sham-OBx hamsters, and the completeness of the OBx procedure was calculated by dividing the amount of remaining OB tissue by the grand mean OB mass.

4.8. Statistical analyses

Effects of photoperiod and bulbectomy on all dependent variables were assessed using 2 (LD, SD) $\times$ 2 (OBx, Sham) factorial ANOVAs. Because photoperiod affected food and saccharin intake prior to injection treatments, changes in food and saccharin intake following LPS and saline injections were each expressed as a percentage of individual baseline values (mean of the three daily measurements immediately preceding the injection). Values were then compared between groups using 2 (LD, SD) $\times$ 2 (OBx, Sham) $\times$ 2 (LPS, Saline) ANOVA, according to the methods of Bilbo et al. (2002b).

Effects of the order of injection treatments on change in food and saccharin intake were evaluated with ANOVA. No significant effect of injection order was observed ($P > 0.05$ in all comparisons), therefore injection groups were collapsed within treatment across the counterbalanced blocks for all analyses (cf. (Prendergast et al., 2008a; Wen et al., 2007)).

All statistical calculations were conducted using Statview 5.0 (SAS Institute, Cary, NC). Where permitted by significant F statistics, pairwise comparisons were conducted using Fisher's PLSD tests. Differences were considered statistically significant if $P < 0.05$.

4.9. Analyses of effect sizes

To compare the magnitude of photoperiodic modulation of several endpoints under intact and OBx conditions, omega square (ω^2_A) values were calculated for each dependent variable. In a single-factor design ω^2_A can be estimated from two variances in the treatment population, one based on the differences between the population treatment means ($\sigma^2_{\text{Treatment}}$), and the other based on the variability within each population ($\sigma^2_{\text{Residual}}$) as follows (Keppel, 1991; Eq. 4-1):

$$\omega^2_A = \sigma^2_{\text{Treatment}} / [\sigma^2_{\text{Treatment}} + \sigma^2_{\text{Residual}}]$$

$\omega^2_A = 0$ when treatment effects are absent, and varies from 0 to 1.0 when treatment effects (in this case photoperiod) are present. Effect magnitude, or strength, as estimated by ω^2_A , is a relative measure reflecting the proportional amount of the total population variance that is attributable to variation between treatment groups. ω^2_A differs qualitatively from an F statistic because it is insensitive to changes in sample size (Carroll and Nordholm, 1975; Lane and Dunlap, 1978). This index is often referred to as the proportion of variation “accounted for” by the experimental manipulation, or “explained variance”. Based on meta-analyses of behavioral data, ω^2_A values less than 0.06 are considered “small”, less than 0.15 are considered “moderate”, and ω^2_A values greater than 0.15 are considered “large” (Cohen, 1977; Cooper and Findley, 1982).

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Fig. 1. Mean ($\pm$ SEM) concentrations of (A) blood leukocytes and (B) plasma cortisol of sham-operated and bilaterally olfactory bulbectomized (OBx) adult male Siberian hamsters following exposure to either long-day (LD, 15 h light/day) or short-day (SD, 9 h light/day) photoperiods for 8 weeks. * $P < 0.05$ between LD and SD; ** $P < 0.05$ vs. LD-sham.

Fig. 2.

Mean ($\pm$ SEM) nocturnal home-cage locomotor activity of sham-operated and OBx adult male Siberian hamsters housed in either long-day (LD, 15 h light/day) or short-day (SD, 9 h light/day) photoperiods. Activity measurements were obtained 6–10 weeks after the onset of experimental photoperiod treatments. * $P < 0.05$ between Sham and OBx.

Fig. 3.

Mean ($\pm$ SEM) baseline (A) 24 h food intake, (B) 3 h saccharin intake, and (C) body mass of sham-operated and OBx adult male Siberian hamsters following exposure to either long-day (LD, 15 h light/day) or short-day (SD, 9 h light/day) photoperiods for 10 weeks. For all panels: $**P < 0.05$ vs. LD-sham value; $##P < 0.05$ vs. SD-sham value.

Fig. 4.

Mean ($\pm$ SEM) percent change in daily food intake (relative to baseline intake) of adult male Siberian hamsters following i.p. injection with 0.625 μ g/kg of bacterial lipopolysaccharide (LPS) or sterile 0.9% saline (Saline; injections delivered on day 0). Prior to injection treatments, hamsters were either sham (OB-intact, top panel) or bilaterally olfactory bulbectomized (OBx, bottom panel) and then housed in either long days (LD) or short days (SD) for 10–12 weeks. Within each panel: * P < 0.05 vs. saline in the same photoperiod; # P < 0.05 vs. SD-LPS value. Across panels: + P < 0.05 vs. SD-sham-LPS value on the same post-treatment day.

Fig. 5.

Mean ($\pm$ SEM) percent change in 3 h nocturnal consumption of 0.1% sodium saccharin (relative to baseline intake) of adult male Siberian hamsters following i.p. injection with 0.625 μ g/kg of bacterial lipopolysaccharide (LPS) or sterile 0.9% saline (Saline; injections delivered on day 0). Prior to injection treatments, hamsters were either sham (OB-intact, top panel) or bilaterally olfactory bulbectomized (OBx, bottom panel) and then housed in either long days (LD) or short days (SD) for 10–12 weeks. Within each panel: * $P < 0.05$ vs. saline in the same photoperiod.

Fig. 6. Mean ($\pm$ SEM) percent change in body mass (relative to baseline) of adult male Siberian hamsters following i.p. injection with 0.625 μ g/kg of bacterial lipopolysaccharide (LPS) or sterile 0.9% saline (Saline; injections delivered on day 0). Prior to injection treatments, hamsters were either sham (OB-intact, top panel) or bilaterally olfactory bulbectomized (OBx, bottom panel) and then housed in either long days (LD) or short days (SD) for 10–12 weeks. Within each panel: # $P < 0.05$ vs. SD-LPS value.

Table 1

Post hoc omega square (ω^2_A) estimates of photoperiod effects on leukocyte counts and LPS-induced changes in behavior and body mass in sham-operated and OBx hamsters

	Sham-operated	OBx
Total leukocytes	0.427	0.124
Food intake	0.194	0 ^a
Saccharin intake	0.078	0 ^a
Body mass	0.285	0 ^a

See Experimental procedures for calculations.

^a Negative ω^2_A values can be obtained when the relevant *F*-statistic is <1.