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Short photoperiods attenuate central responses to an inflammogen

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ABSTRACT

In most parts of the world, environmental conditions vary in a predictable seasonal manner. Thus, seasonal variation in reproductive timing and immune function has emerged in some species to cope with disparate seasonal demands. During the long days of spring and summer when food availability is high and thermoregulatory demands low, Siberian hamsters invest in reproduction, whereas during the harsh short days of winter hamsters divert energy away from reproductive activities and modify immune capabilities. Many seasonal adaptations can be recapitulated in a laboratory setting by adjusting day length (photoperiod). Early-life photoperiods are important sources of seasonal information and can establish an individual's developmental trajectory. Siberian hamsters housed under short days (SD; 8 h light/day) recover more rapidly than long-day (LD; 16 h light/day) hamsters from immune activation with lipopolysaccharide (LPS). SD hamsters attenuate fever response, reduce cytokine production, and abrogate behavioral responses following LPS injection. The mechanism by which SD Siberian hamsters attenuate febrile response remains unspecified. It is possible that periphery-to-brain communication of inflammatory signals is altered by exposure to photoperiod. Rather than testing photoperiod effects on each of the multiple routes by which immunological cues are communicated to the CNS, we administered LPS intracerebroventricularly (i.c.v.) following adolescent exposure to either 6 weeks of SD or LD. Injection of LPS i.c.v. led to a similar immune reaction in SD hamsters as previously reported with intraperitoneal injection. Short days attenuated the response to LPS with diminished fever spike and duration, as well as decreased locomotor inactivity. Furthermore, only LD hamsters demonstrated anhedonic-like behavior following LPS injection as evaluated by decreased preference for a milk solution. These results suggest that photoperiodic differences in response to infection are due in part to changes in central immune activation.

1. Introduction

Many non-tropical animals display annual variation in reproductive and immune activity. Winter represents an energetic bottleneck during which increased thermoregulatory requirements coincide with reduced energy availability (Martin et al., 2008). During the spring and summer, which encompasses much of the breeding season for small, nontropical vertebrates, investments are biased towards reproductive activities; however, during late autumn and winter, energy is reallocated to mechanisms promoting over-winter survival. Short day lengths (SD; <12.5 h light/day) inhibit reproductive function, and alter several aspects of immune function in some seasonally breeding rodents (Bilbo et al., 2002a; Demas et al., 1996; Goldman, 2001; Navara et al., 2007; Prendergast et al., 2003a,b, 2007a; Weil et al., 2006a). Inflammatory responses, in particular, are modulated by photoperiod (Bilbo et al., 2002b; Fenn et al., 2011; Prendergast et al., 2003a; Pyter et al., 2005). One especially well-characterized short-day effect on immune function is that adult Siberian hamsters (*Phodopus sungorus*) recover faster than long-day hamsters from immune activation to lipopolysaccharide (LPS; a component of gram negative bacterial cell walls that activates the immune system; AKA endotoxin) (Bilbo et al., 2002b).

Because environmental conditions vary in a predictable seasonal manner over most of the planet, the external conditions experienced during early development differ among individuals born at different times of the year. Siberian hamsters use photoperiod information early in life, as well as in adulthood to establish somatic and behavioral developmental trajectory (Pyter and Nelson, 2006; Weil et al., 2006b). Although much of the research on photoperiod effects on immune function is based on adults, early-life photoperiod is an important source of seasonal information (Prendergast et al., 2004a; Pyter and Nelson, 2006; Weil et al., 2006b). Because the adaptations associated with investing in reproduction or survival mechanisms are generally mutually exclusive, it is critical for small animals to respond appropriately to seasonal cues soon after birth.

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Fever and sickness behavior are ubiquitous phenomena in vertebrate physiology. Understanding of the physiological and behavioral consequences of innate immune activation has relied on studies of the administration of the inflammogen, LPS. Animals treated with LPS display a coordinated suite of physiological and behavioral responses (Exton, 1997). LPS is first detected by Toll-like receptors (TLRs; for review see: Akira and Takeda, 2004) expressed on a variety of cell types in the periphery. The activation of TLRs, by heterodimer of TLR4 and myeloid differentiation factor 2 for LPS responses (Park et al., 2009), induces both the activation of the nuclear factor kappa B signaling cascade and the production of endogenous signaling factors. The primary mediators of the sickness response are the proinflammatory cytokines, interleukin-1β (IL-1 β), IL-6, and tumor necrosis factor-α (TNFα) (Dantzer, 2001; Kentner et al., 2010).

The behavioral sequelae of endotoxin administration are particularly salient and include lethargy, anorexia, adipsia, anhedonia and reduced social interactions (Hart, 1988). These responses collectively termed 'sickness behavior,' along with the induction of fever, are thought to be part of a coordinated, adaptive effort to aid in recovery from infection (Hart, 1988; Kentner et al., 2010). Intraperitoneal LPS injections induce cytokine gene expression, in both the periphery and the CNS. Although the precise mechanisms remain under investigation, peripheral cytokines are thought to induce central production of cytokines via active transport of cytokine proteins across the blood–brain-barrier (BBB) (Gutierrez et al., 1993), interaction with receptors on epithelial cells, activation of vagal afferents (Ek et al., 1998; Goehler et al., 1999), and by diffusion into circumventricular brain regions (Quan et al., 1998). Expression of cytokines in the brain appears to underlie the behavioral effects of peripheral LPS administration (Laye et al., 2000). In the brain, cytokines are largely expressed by microglia and perivascular and meningeal macrophages (van Dam et al., 1992).

Both *in vivo* and *in vitro* responses to LPS are reduced by exposure to short days (Prendergast et al., 2003a; Pyter and Nelson, 2006; Pyter et al., 2005; Weil et al., 2006b). Attenuated febrile and behavioral responses to LPS in short-photoperiod hamsters (Romanovsky et al., 2005) are associated with reduced neural cytokine gene expression and peripheral cytokine production. In this study we investigate how pre-pubertal photoperiod alters neuroimmune responses to central inflammation. More specifically, we tested the hypothesis that periphery-to-brain communication of inflammatory signal is altered by exposure to different photoperiods. If Siberian hamsters do not show the typical pattern of immune activation following i.c.v. injection, then these results would suggest that photoperiodic differences in inflammatory response are not driven by changes in the CNS. Whereas if similar behavioral and febrile responses occur following central LPS administration, then these results would suggest that disparate results to immune stimulation in SD and LD are in part due to central alterations in response to the inflammogen.

Excessive or septic levels of LPS can compromise the BBB and permit infiltration of immune cells from the periphery into the brain (Laflamme and Rivest, 2001). For this reason, we administered a low dose of LPS (2.5 μg) i.c.v. to induce a mild fever and sickness response. Injection of LPS i.c.v. caused a similar fever response in SD hamsters as previously reported with intraperitoneal injection. Short days diminished fever response following central LPS administration. SD hamsters also reduced sickness behavioral responses; SD hamsters blunted LPS-induced inactivity and decreased anhedonic-like behaviors compared to LD hamsters. These finding indicate that photoperiodic differences in response to infection are due in part to changes in central immune activation.

2. Methods

2.1. Animals

Male Siberian hamsters (*P. sungorus*) used in this study were bred in our colony at the Ohio State University from a wild-bred stock obtained from Dr. K. Wynne-Edwards (Kingston, Ontario, Canada). Hamsters were housed in polypropylene cages (28 × 17 × 12 cm) with a nestlet and 1 cm of corncob bedding. All hamsters had *ad libitum* access to food (Harlan Teklad Rodent Diet 8640, Indianapolis, IN, USA) and filtered tap water. Animal rooms were held at constant temperature and humidity (21 ± 2 °C and 50 ± 10%, respectively). All procedures were conducted in accordance with the US National Institute of Health (1986) Guide for the Care and Use of Laboratory Animals and the Ohio State University Institutional Animal Care and Use Committee.

All hamsters were born into long photoperiod (16L:8D; lights off 15:00 EST; LD). Immediately after weaning at 21–24 days of a age, a subset of hamsters were transferred to short photoperiod (8L:16D; lights off 15:00 EST; SD) while the remaining hamsters were maintained in LD. All hamsters were weighed upon entrance into the study and weekly for the duration of the experiment in order to monitor photoperiodic responsiveness.

2.2. Surgery

After 5 weeks in photoperiod conditions, all hamsters underwent two procedures in a single session. First, a guide cannula was inserted, targeting the left lateral ventricle. The hamsters were anesthetized with 1–1.5% isofluorane in oxygen-enriched air and were placed in a stereotaxic apparatus (David Kopf Instruments). An incision was made along the midline to locate Bregma. The cannula, cut 2.35 mm below the pedestal, was positioned at +0.1 mm posterior and +0.9 mm lateral to bregma and secured with glue (Detillion et al., 2004). Once the glue was dry, a dummy cannula was inserted into the guide cannula. Directly following cannulations hamsters were maintained on isoflurane vapors and implanted i.p. with radio-telemetric transmitters (Mini-Mitters) (Castanon et al. 2001; Bilbo et al. 2002b). Following surgeries hamsters were administered 0.75 μg i.p. injection of buprenorphine in sterile saline, placed in a clean home cage, and constantly monitored for 2 h in case complications occurred.

2.3. Fever assessment

Following a 1 week recovery (during week 6), hamsters received three consecutive injections 24 h apart. Hamsters were injected with sterile artificial cerebral spinal fluid (aCSF) for two days and then LPS (2.5 μg LPS from Escherichia coli serotype 026:B6, Sigma Chemical) in 2 μL sterile aCSF) (Castanon et al. 2001; Wen and Prendergast, 2007). Animals were either used for fever assessment (SD $n = 7$; LD $n = 8$) or milk consumption ($n = 5$ per group). For hamsters used in fever assessment, upon return to the animal room following surgery, cages were placed on receivers (Mini-Mitter) connected to a computer. Receivers collated emitted body temperature and activity frequencies continuously over 30 min intervals and converted them to raw data based on pre-programmed calibration curves for each transmitter. For four days prior to LPS injection (including two days of aCSF injections) baseline body temperature and activity were monitored. Ten days after telemeter implantation, hamsters were injected i.c.v. with LPS dissolved in sterile aCSF. Injections occurred at 15.00 h, just prior to lights out. Body temperature and activity were monitored over the next 24 h. For hamsters that underwent testing with the milk

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solution, for four days prior to the LPS injections, food intake and consumption of sweetened condensed milk solution (Kroger brand, diluted 1:1 with tap water) was recorded. This milk solution is readily consumed by rodents and lack of consumption is operationally defined as anhedonic behavior indicating sickness. For 4 h each night (15.00–19.00 h), food and water bottles were removed, and a modified bottle containing 10 mL diluted milk was placed in the cage. Milk intake was quantified by subtracting the mass of the syringe at the end of the session from its initial filled mass. Food and milk intake were monitored for 48 h following LPS injections. Hamsters were killed 48 h post-LPS via decapitation while under deep isoflurane anesthesia, and tissue was collected and weighed.

2.4. Statistical analyses

Main effects of photoperiod condition (LD, SD) were assessed using a one-way analysis of variance (ANOVA). Baseline body temperatures and locomotor activity for the active and inactive phases were determined for each hamster using the mean values for the 2 days of aCSF injections before LPS. Fever was assessed using a repeated measures ANOVA with photoperiod as the between subjects factor and time the within subjects factor. For fever duration, we defined fever as temperatures significantly ($p < 0.05$) higher than active-phase baseline for each 30-min interval using two-tailed *t*-tests. Mean total locomotor activity for the 8 h following LPS treatment is expressed as change from baseline and percent change from baseline activity (taken 24 h prior) and analyzed using a one-way ANOVA. Mean differences were considered statistically significant when $p < 0.05$. All statistical analyses were conducted using SAS 9.2 software.

3. Results

3.1. Somatic measures

There were no differences in body mass or estimated testes volume between groups upon placement into photoperiod ($p > 0.05$). At the conclusion of the study, SD hamsters significantly reduced body mass as compared to LD hamsters ($F_{1,13} = 12.93$; $p < 0.005$; Fig. 1A). SD hamsters displayed lower reproductive tissue mass; SD hamsters reduced paired testes mass, epididymal fat pads mass, seminal vesicles mass, and epididymides mass ($F_{1,13} = 197.66$, 50.92, 65.99, and 76.57, respectively; $p < 0.0001$; Table 1). Pelage score was elevated among SD hamsters, indicating greater whitening of the fur ($F_{1,13} = 62.70$; $p < 0.0001$; Fig. 1B). One SD non-responder, defined in photoperiodic rodents as not reducing testes mass to two standard deviations below the LD mean (Desjardins and Lopez, 1983), was excluded from comparisons.

Table 1
Reproductive tissue weights in milligrams. Expressed as mean ± SEM.

	Long day	Short day
Paired testes mass	637 ± 39	44 ± 4*
Epididymal fat pads mass	756 ± 78	137 ± 23*
Seminal vesicles mass	89 ± 8	14 ± 4*
Epididymides mass	162 ± 9	49 ± 9*

* $p < 0.0001$.

3.2. Fever

Baseline body temperature did not differ between photoperiods ($p > 0.05$; data not shown). LPS significantly increased body temperature in both SD and LD hamsters ($F_{24,672} = 6.989$; $p < 0.0001$). SD hamsters reduced febrile response over time compared to LD hamsters ($F_{24,672} = 2.310$; $p < 0.001$; Fig 2A). Fever was defined as significantly higher temperature than after aCSF injection. Maximum fever amplitude did not differ between photoperiods ($p > 0.05$). However, duration of fever, was attenuated among SD hamsters ($F_{1,13} = 13.128$; $p < 0.05$; Fig 2B).

3.3. Activity

Hamsters decreased locomotor activity following LPS injection. However, LD hamsters had greater reductions in locomotor activity than SD hamsters following LPS injection ($F_{1,13} = 4.64$; $p < 0.05$). LD hamsters also had greater percent reduction in locomotor activity than SD hamsters following LPS injection ($F_{1,13} = 4.57$; $p = 0.05$; Fig 3A).

3.4. Milk intake

There were no differences between photoperiods with respect to baseline milk consumption ($p > 0.05$). Following LPS injection LD hamsters significantly reduced milk consumption as compared to SD hamsters ($F_{2,16} = 5.037$; $p < 0.05$; Fig 3B). Furthermore, there was an enduring reduction in milk consumption among LD hamsters; LD hamsters also reduced milk consumption compared to SD hamsters on the day following LPS injection.

4. Discussion

Winter is a particularly difficult time for animals to survive and reproduce. Proper allocation of energy to promote survival is an important adaptation among many seasonally breeding species. Mounting an immune response is energetically costly. Furthermore, over-activation on the immune system can result in lethal endotoxemia (Prendergast et al., 2003a). As such, Siberian

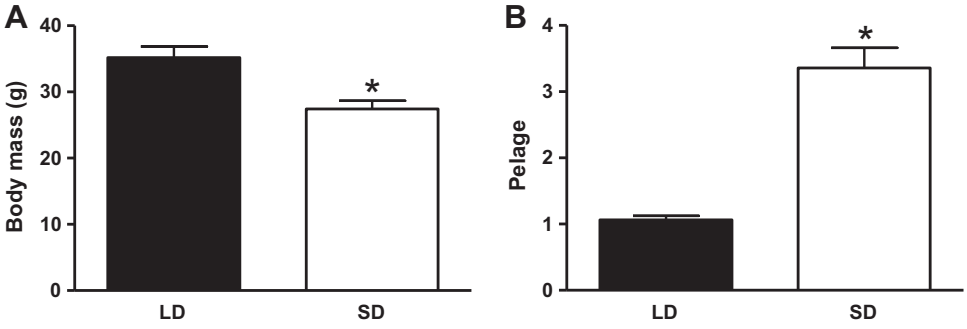

Fig. 1. (A) Body mass prior to Mini-Mitter implants. (B) Pelage value at conclusion of the study. For pelage, values range from 1 (LD brown coloration) to 4 (SD white coloration). Data are expressed as mean ± standard error of the mean [SEM]; *$p < 0.05$ between groups.

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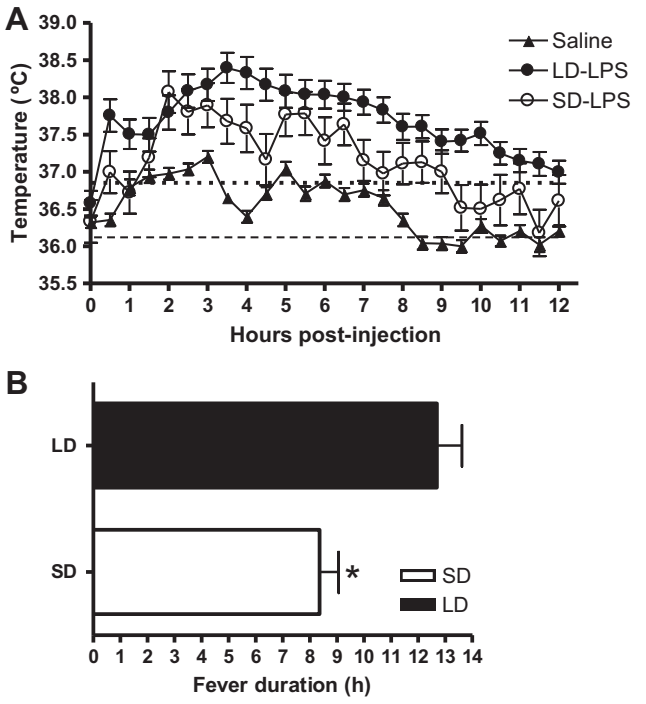

Fig. 2. (A) Body temperature from 0 to 12 h post-LPS injections in LD (black circles) and SD (open circles) hamsters, and after saline injections (triangles). Horizontal dashed and dotted lines represent mean baseline body temperature during the inactive (36.1 °C) versus active (36.6 °C) phases, respectively. (B) Duration (h) of body temperatures higher than the active baseline in LD and SD hamsters following LPS injections. Data are expressed as mean ± SEM; $^{*}p < 0.05$ between groups.

hamsters have developed a coordinated suite of immunological changes, in response to short photoperiods that results in both enhancement and suppression of different aspects of the immune system. For example, SD hamsters elevate the concentration of circulating leukocytes, such as natural killer cells and blood lymphocytes, while suppressing basal lymphocyte cell proliferation (Bilbo et al., 2002b; Demas et al., 1996; Demas and Nelson, 1998; Prendergast et al., 2001). Behavioral and febrile response to LPS are attenuated in SD which may protect against lethal bacterial sepsis (Bilbo et al., 2002b; Demas et al., 1996; Nelson et al., 2002; Owen-Ashley and Wingfield, 2007; Prendergast et al., 2004b), whereas delayed-type hypersensitivity (DTH) responses are enhanced which may increase resistance to fungi and microbes (Bilbo et al., 2002a; Goldman, 2001; Nelson et al., 2002; Prendergast et al., 2003a; Vitale et al., 1985). These modifications allow for increased immune competence without dedicating physiological resources to energetically expensive processes. Although many different aspects of the immune system have been characterized in the context of photoperiod, little is known about how day length affects the integrated function of these components. The mammalian immune system is highly complex and an understanding of the mechanisms through which photoperiod modulates immune function is incomplete.

Attenuated fever has previously been reported among SD Siberian hamsters after intraperitoneal administration of LPS (Bilbo et al., 2002b). However, it was unclear whether differences in photoperiodic response to infection were primarily due to differential response to the inflammogen or changes in the afferent immune pathways from the periphery to the brain. Here we show that hamsters housed under short photoperiods reduce febrile response following i.c.v. administration of LPS as compared to LD conspecifics (Fig. 2). These results are significant because they indicate that direct central immune activation cause an attenuated sickness response in SD hamsters. This supports the premise that a differential response within the brain of SD hamsters accounts for at least part of the attenuated fever response and cytokine profile induced by systemic immune activation.

Suppression of pro-inflammatory cytokine production by immune cells in SD hamsters may be the mechanism of attenuated fever response. For example, microglia (centrally) or macrophages (peripherally) may reduce fever response in SD by suppressing cytokine production in the CNS or periphery, respectively. Macrophages/microglia mediate LPS signaling through TLR4 and TLR2 and are important in alerting other cells to immune challenge (Laflamme and Rivest, 2001; Olson and Miller, 2004). SD macrophages produce less TNF than LD macrophages when stimulated with LPS *in vitro*; therefore, short days inhibit macrophage response to pathogen associated molecular patterns. There are however, no differences in TLR2 or TLR4 gene expression on these macrophages. This suggests inflammogens may bind to the ligand with different affinities or trigger downstream effects differently (Navara et al., 2007). Alternatively, other cell populations may be responsible for photoperiod differences in immune response. The vast majority of cytokines that access the brain with peripheral LPS stimulation come from endothelial cells that relay the inflammatory signal (Verma et al., 2006). Thus, changes in endothelial responsiveness may play a role in photoperiod differences to LPS. With i.c.v. administration endothelial cells may still have contact with LPS and mediate central differences in photoperiod response (Verma et al., 2006).

Understanding the glial response to photoperiod is important because of their potential role in propagating inflammatory signal after activation of the peripheral nervous system. Our results suggest that short days are associated with decreased reactivity of immune cells in the CNS. The results do not rule out an independent attenuation of the inflammatory response in the periphery as well. Short photoperiods have previously been reported to downregulate

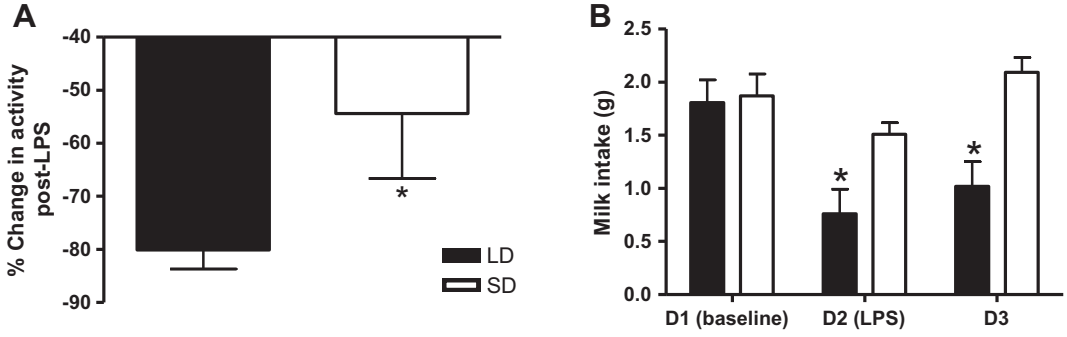

Fig. 3. (A) Decrease in activity compared to baseline on the day of LPS injection. (B) Sweetened condensed milk intake on the day prior to, day of, and day following LPS injection. Data are expressed as mean ± SEM; $^{*}p < 0.05$ between groups.

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cytokine production in peripherally extracted macrophages as well as the central nervous system following i.p. LPS administration (Bilbo et al., 2002b). It is possible that peripheral photoperiodic differences in immune response are communicated back to the periphery following CNS immune activation. For example, in the brains of aged rodents, heightened immune reactions are communicated back to the periphery following i.c.v. LPS injection. Aged mice demonstrate elevated plasma IL6 concentrations as compared to adult mice following central administration of LPS (Huang et al., 2008). Importantly, LPS and other innate immune activators do not typically gain direct access to the brain (Bechmann et al., 2007). Although minute amounts of LPS (<0.025% of an injection) cross the murine BBB; the amount is insufficient to stimulate the brains neuroimmune response alone (Banks and Robinson, 2010). Therefore, independent of adequate levels of direct contact with LPS, the sickness response is partially abrogated in short days. This further implicates other cell populations such as endothelial cells in modulating photoperiod responses (Bilbo et al., 2002b). Glia may become activated to a lesser extent in SD hamsters following LPS exposure, producing decreased amount of inflammatory mediators such as IL1β, TNFα, and IL6. Alternatively, SD hamsters may produce larger amounts of anti-inflammatory mediators or growth factors such as IL4, IL10, or IGF1 resulting in a more rapid resolution of the inflammatory response.

Extended elevation of inflammatory cytokines is associated with prolonged demonstration of sickness behaviors (Dantzer, 2001). Furthermore, peripheral LPS administration is associated with increased anhedonia and reduced activity in SD as compared to LD hamsters (Wen and Prendergast, 2007). In this study, LD, but not SD hamsters, significantly reduced milk consumption immediately following LPS administration. 24 h following LPS challenge, LD hamsters maintained low milk consumption, whereas SD hamsters continued to drink pre-LPS levels of milk (Fig. 3). Similarly, SD hamsters did not reduce locomotor activity as much as LD hamsters following LPS injection. These reductions in sickness behaviors are further indicative of a decreased propagation of the inflammatory signal in SD hamsters.

Short photoperiods may alter immune function in Siberian hamsters through direct and indirect actions of melatonin on the immune system as well as through secondary mediators (such as steroid hormones). Microglia, the primary immune cells of the CNS possess both MT1 and MT2 receptors (although in Siberian hamsters MT2 receptors are not functional (Weaver et al., 1996; Olivier et al., 2009)). The effects of melatonin on microglia *in vitro* are disparate (Shafer et al., 2001). In contrast, *in vivo* melatonin generally has an anti-inflammatory role producing decreased microglial activation (Wu et al., 2011; Zhou et al., 2008). Astrocytes, which also possess melatonin receptors, similarly appear to reduce activation and cytokine release following melatonin administration (Lee et al., 2010). Photoperiodic differences in immune activation can be recapitulated by chronic, timed administration of melatonin. One week of short photoperiod-like melatonin however, does not mimic seasonal changes in immune function which supports an indirect role of melatonin on photoperiod induced changes in immune function (Bilbo and Nelson, 2002). Melatonin may work through secondary mediators such as steroid hormones. For example, castration alters immune responses in Siberian hamsters, although photoperiodic effects on immune response persist even in castrated hamsters (Prendergast et al., 2007a).

In conclusion, i.c.v. LPS injection leads to a similar immune reaction in SD hamsters as previously reported with intraperitoneal injection (Bilbo et al., 2002b). These findings indicate that desensitization of the inflammatory signal in SD is occurring within the brain. The mechanisms by which photoperiodic differences in immune response are produced have yet to be established. For example, it is possible that the sensitivity of the cells expressing TLRs that are principally responsible for the detection of endotoxin is reduced in short-day hamsters. Alternatively, photoperiod induced differences in hypothalamic–pituitary–adrenal axis function may affect immune function.

Overall, external environment is not static. Animals use environmental information to establish physiological priorities and regulate development. Although this study focuses on a single environmental factor, it has implications for a wide variety of early life experiences that can act to shape adult phenotypes. Understanding the seasonal influences on immune function is important in order to predict emergence of new diseases, as well as predict changes in the onset of established diseases in the context of altered environments. Additionally an understanding of the specific mechanisms that mediate fluctuations in endotoxin responsiveness can potentially provide insight into other conditions where acute phase responses are altered, including invasive and migratory species and disrupted seasonality associated with light pollution (Lee and Klasing, 2004; Navara and Nelson, 2007).

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