Chronobiology International, 29(1): 26–34, (2012)

ISSN 0742-0528 print/1525-6073 online
DOI: 10.3109/07420528.2011.635831

informa
healthcare

Dim Light at Night Increases Immune Function in Nile Grass Rats, a Diurnal Rodent

Laura K. Fonken, Achikam Haim, and Randy J. Nelson

Department of Neuroscience and Institute for Behavioral Medicine Research, The Ohio State University, Columbus, Ohio, USA

With the widespread adoption of electrical lighting during the 20th century, human and nonhuman animals became exposed to high levels of light at night for the first time in evolutionary history. This divergence from the natural environment may have significant implications for certain ecological niches because of the important influence light exerts on the circadian system. For example, circadian disruption and nighttime light exposure are linked to changes in immune function. The majority of studies investigating the effects of light exposure and circadian disruption on the immune system use nocturnal rodents. In diurnal species, many hormones and immune parameters vary with secretion patterns 180° out of phase to those of nocturnal rodents. Thus, the authors investigated the effects of nighttime light exposure on immunocompetence in diurnal Nile grass rats (*Arvicanthis niloticus*). Rats were housed in either standard 14-h light (L):10-h dark (D) cycles with L ~150 lux and D 0 lux or dim light at night (dLAN) cycles of LD 14:10 with L ~150 lux and D 5 lux for 3 wks, then tested for plasma bactericidal capacity, as well as humoral and cell-mediated immune responses. Rats exposed to dLAN showed increased delayed-type hypersensitivity pinna swelling, which is consistent with enhanced cell-mediated immune function. dLAN rats similarly showed increased antibody production following inoculation with keyhole lymphocyte hemocyanin (KLH) and increased bactericidal capacity. Daytime corticosterone concentrations were elevated in grass rats exposed to nighttime dim light, which may have influenced immunological measures. Overall, these results indicate nighttime light affects immune parameters in a diurnal rodent. (Author correspondence: fonken.1@osu.edu)

Keywords: *Arvicanthis niloticus*, Bacteria killing, Delayed-type hypersensitivity, Grass rats, Keyhole lymphocyte, Light at night

INTRODUCTION

Most organisms possess an endogenous biological clock that is synchronized by a very reliable exogenous cue: the daily cycle of light (L) and dark (D) produced by the rotation of the Earth about its axis. This biological clock is adaptive as it helps to maintain both daily and seasonal rhythms that allow animals to anticipate changes in the external environment (Hut & Beersma, 2011). The natural LD cycle, however, is now disrupted for many humans and nonhuman animals. With the advent of electrical light, light exposure is no longer limited to the natural pattern. Instead of aligning the circadian system with a stable cyclical factor, individuals currently experience a variety of lighting schedules. This divergence from the natural environment is not without repercussions. Disruptive lighting affects many physiological and behavioral functions (Fonken & Nelson, 2011; Navara & Nelson, 2007). For example, individuals exposed to altered light cycles are at increased risk for heart disease (Ha & Park, 2005); cancer (Davis & Mirick, 2006; Kloog et al., 2011; Schernhammer et al., 2001; Stevens et al., 2011); sleep (Deboer et al., 2007; Kohyama, 2009; Martinez-Nicolas et al., 2011), mood (Dumont & Beaulieu, 2007; Fonken et al., 2009; Parry et al., 2010), metabolic (Fonken et al., 2010; Reiter et al., 2011), and reproductive (Fiske, 1941; Thomas et al., 2001) disorders; plus circadian rhythm alterations (Borugian et al., 2005) and disrupted neuroendocrine rhythmicity (Claustrat et al., 2008; Persengiev et al., 1991). One common factor across many of these pathologies is altered immune function.

Circadian timing in mammals is organized by a hierarchy of oscillating tissues, at the top of which are the suprachiasmatic nuclei (SCN) of the hypothalamus (Reppert & Weaver, 2002). Light information is the primary entraining cue for this master circadian clock. Light travels from the external environment through the intrinsically photosensitive retinal ganglion cells (ipRGCs) to the SCN. The SCN then influences downstream "slave" oscillators via the autonomic nervous system and control of the sleep-wake cycle. SCN-driven

Submitted September 2, 2011, Returned for revision September 24, 2011, Accepted October 15, 2011

Address correspondence to Laura K. Fonken, Department of Neuroscience, The Ohio State University, 460 W. 12th Avenue, Columbus, OH 43210, USA. Tel.: 614-688-4674; Fax: 614-688-4733; Email: fonken.1@osu.edu

sympathetic innervation of the pineal gland regulates the release of melatonin; nighttime sympathetic neural stimulation leads to the production of melatonin from its precursor serotonin in the pineal gland. This nocturnal melatonin signal provides time-of-day information to cells throughout the body and is the most reliable peripheral marker of central clock activity (Blask, 2009). Nocturnal lighting, if sufficiently bright, disrupts the synthesis of melatonin (Brainard et al., 1982, 1985; Dauchy et al., 2010). Importantly, modulation of both the circadian system and melatonin alters immunological measures.

Multiple immune markers, such as interleukin 2 (IL2), IL10, IL6, IL1β, tumor necrosis factor alpha (TNFα), interferon gamma (IFNγ), and chemokine receptor 2 (CCR2), are expressed in a circadian pattern (Lundkvist et al., 1998; Young et al., 1995). Disruption of the circadian system through rapid transmeridian travel (i.e., jet-lag), shiftwork, genetic mutations, or atypically timed light exposure changes the normal pattern of immune parameters. For example, mice with a loss of function mutation in the clock gene *Period 2* (*Per2*) have irregular production of IL10 and IFNγ in response to lipopolysaccharide (LPS) injection (Arjona & Sarkar, 2006). Mice deficient in *Bmal1*, another critical clock component, show early signs of aging, such as sarcopenia, cataracts, organ shrinkage, and elevated reactive oxygen species (ROS) in the kidney and spleen. The life spans of *Bmal1*-deficient mice are also reduced (Kondratov et al., 2006). *Cry* 1 and 2 knockout mice have exacerbated cytokine and joint swelling after arthritic induction (Hashiramoto et al., 2010). Furthermore, a phase-advancing chronic jet-lag (CJL) protocol causes persistent hypothermia and reduced survival following LPS administration in mice, and macrophages extracted from these mice have increased cytokine response to LPS (Castanon-Cervantes et al., 2010). Finally, rotating shiftworkers periodically exposed to light at night have increased leukocyte count and C-reactive protein concentrations (Puttonen et al., 2011).

The nighttime increase in pineal melatonin production and secretion correlates with reduced innate immune responses (Markus et al., 2007). Nuclear factor kappa B (NF-κB), a pleiotropic transcription factor involved in the regulation of genes encoding for immune-related enzymes, displays daily variation (Cecon et al., 2010; Chen et al., 1999). NF-κB induces multiple proinflammatory cytokines, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2). Melatonin blocks NF-κB nuclear translocation in leukocytes and endothelial cells, suppressing immune-gene transcription activity (Gilad et al., 1998; Tamura et al., 2009). Darkness-induced suppression of NF-κB transcription is blocked by propanolol (a drug that inhibits melatonin production in addition to altering catecholaminergic function). In vitro work has further supported the role of melatonin in NF-κB signaling, as melatonin reduces NF-κB in cultured pineal glands (Cecon et al., 2010). In a rat model of diabetes, increased expression of NF-κB and proinflammatory cytokines, including TNFα and IL6, were reduced with melatonin treatment (Negi et al., 2011). Melatonin also impairs capacity for rolling and adhesion among leukocytes (Lotufo et al., 2001). Furthermore, communication between the circadian and immune system is bidirectional, with multiple studies characterizing an immune-pineal axis (Couto-Moraes et al., 2009; Lopes et al., 2001; Markus et al., 2007). For example, administration of LPS inhibits norephinephrine (NE)-induced *N*-acetylserotonin (NAS) production, resulting in decreased nocturnal melatonin production (da Silveira Cruz-Machado et al., 2010).

Taken together, these studies suggest that nighttime lighting may affect immune function through disruption of the circadian system and suppression of melatonin. Recently, our laboratory demonstrated that Siberian hamsters (*Phodopus sungorus*) exposed to dim nighttime lighting (dLAN) have reduced immunological capabilities (Bedrosian et al., 2011b). Housing female Siberian hamsters under dLAN for 4 wks reduces delayed-type hypersensitivity responses, decreases bactericidal capacity of blood, and prevents fever-associated reductions in locomotor activity. Because Siberian hamsters are nocturnal, and in the laboratory are typically exposed to light at night (LAN) during their active period, we asked whether LAN would evoke similar responses in a diurnal species. Thus, we investigated the effects of nighttime light exposure on immune parameters in Nile grass rats (*Arvicanthis niloticus*), a diurnal rodent species. Male grass rats were exposed to either a standard LD cycle (0 lux light intensity at night) or dim light (5 lux) at night for 3 wks and then tested for delayed-type hypersensitivity, bacteria killing capacity, and antibody production.

METHODS

Animals

Male grass rats (*Arvicanthis niloticus*) used in this study were bred in our colony at the Ohio State University from a wild stock obtained by Dr. Laura Smale, Michigan State University, from the Masai Mara reserve in Kenya. Grass rats were bred under a LD 14:10 cycle, with light of ~150 lux and dark of 0 lux; lights illuminated at 07:00 h Eastern Standard Time [EST]), and all animals were provided food (ProLab RMH 2000; LabDiet, Brentwood, MO) and water ad libitum. Experimental grass rats were weaned between 21 and 24 d of age and housed with same-sex siblings in polypropylene cages (40 cm × 20 cm × 20 cm) with straw bedding. At approximately 3 mo of age, grass rats were singly housed, randomly assigned a number, and were either maintained in LD (n = 9) or placed in dLAN (n = 9 for the remainder of the study). Immunological testing began after 3 wks in the lighting conditions. Colony rooms were maintained at a temperature of 20°C ± 4°C and a relative humidity of 50% ± 10%. All procedures were conducted with approval of the Ohio State University Institutional Animal Care and Use Committee, in compliance with all US federal animal welfare requirements, and

in accordance with the international ethical standards (Portaluppi et al., 2010).

Delayed-Type Hypersensitivity (DTH)

DTH is a cell-mediated response that provides information about the primary immune reaction to invading pathogens. After 3 wks in the respective lighting conditions, grass rats were assessed for DTH response to the chemical antigen 2,4-dinitro-1-fluorobenzene (DNFB; Sigma, St. Louis, MO). Grass rats were individually brought into a procedure room between 14:00 and 15:00 h EST, lightly anesthetized with isoflurane vapors, weighed, and a blood sample was collected from the retro-orbital sinus for use in the bacteria killing and corticosterone assays (see below). Following blood collection, a 1 × 2-cm patch of fur was shaved on the dorsum, and 25 μL of DNFB in a .5% solution (*w*/*v*) of 4:1 acetone to olive oil (prepared fresh daily) was applied to the dorsal skin in the same location on 2 consecutive days. To obtain a baseline measurement, both right and left pinnae were measured during sensitization with a constant loading dial micrometer (Mitutoyo, America Corp., Aurora, IL). Grass rats were then left undisturbed for 1 wk, after which they were again anesthetized, pinna thickness measured, and challenged on the surface of the right pinna with 20 μL of .2% (*w*/*v*) DNFB in 4:1 acetone to olive oil. The left pinna was treated with vehicle solution, and both pinnae were measured every 24 h for 7 days by L.K.F. Pinna swelling values obtained on each day were expressed as a percentage of baseline thickness. All measurements occurred between 14:00 and 15:00 h EST. DTH is an in vivo measure of cell-mediated immune responses that is characterized by swelling at the site of DNFB challenge. Swelling of the right pinna is due to infiltration of leukocytes into the epidermis and dermis (Vadas et al., 1975). This immune measure was previously validated; pinna swelling is positively correlated to the intensity of the immune reaction (Phanuphak et al., 1974).

Keyhole Limpet Hemocyanin (KLH)

Two weeks following the conclusion of DTH measures, humoral immune function was assessed by injecting grass rats with 140 μg KLH suspended in .2 mL sterile saline. KLH is a respiratory protein from the giant keyhole limpet (*Megathura crenulata*) that produces a robust antigenic response without inducing fever or long-term inflammatory response. KLH production has not previously been assessed in grass rats; therefore, this dose was determined based on other arvicoline rodents and resulted in similar patterns of plasma immunoglobulin G (IgG) production (Klein & Nelson, 1999; Weil et al., 2006). Blood was drawn from the retro-orbital sinus at the time of injection and 5, 10, and 15 d post injection in order to capture peak immunoglobulin production (Demas et al., 1997). All blood sampling occurred between 14:00 and 15:00 h EST. Blood samples were centrifuged at 4°C for 30 min at 3.3 × *g*, and plasma was pulled off and stored in microcentrifuge tubes at –80°C.

KLH ELISA

Plasma concentrations of anti-KLH IgG were determined using an enzyme-linked immunosorbent assay (ELISA) as previously described (Demas et al., 1997). Plates were coated overnight with dialyzed KLH antigen, washed, and blocked the subsequent night with a milk-blocking buffer. Plates were then washed, and 150 μL of plasma diluted 1:80 in phosphate-buffered saline (PBS) + Tween was added in duplicate to the wells. Following a 3-h incubation, plates were again washed and incubated for 1.5 h with a secondary antibody (alkaline phosphatase-conjugated anti-mouse IgG). Plates were then treated with the enzyme substrate (*p*-nitrophenyl phosphate) before determining the optical density of each well with a plate reader (Benchmark Microplate Reader; Bio-Rad, Hercules, CA) and 405 nm wavelength filter. To minimize intra-assay variability, optical density was averaged over duplicate wells and expressed as a percentage of the plate-positive control value for statistical analyses.

Bactericidal Capacity of Blood Plasma

Blood samples collected directly prior to DTH sensitization were used in this assay. Blood was centrifuged at 3300 × *g* for 30 min at 4°C, and plasma was pulled off and stored at –80°C. Plasma samples were diluted 1:20 in L-glutamine CO_2-independent media (Gibco, Carlsbad, CA). A standard number of colony-forming units (CFUs) of *Escherichia coli* (Epower 0483E7; Fisher Scientific, Pittsburgh, PA) was added to each sample, and samples were incubated for 30 min at 37°C. Using sterile techniques, 75 μL of each sample were plated in duplicate on tryptic soy agar plate. Two positive controls of diluted bacteria alone and two negative controls of CO_2-independent media were also plated. Plates were inverted and incubated overnight, and total CFUs were counted and expressed as a percent of the positive control.

Radioimmunoassay Procedure (RIA)

Blood samples were collected for RIA of corticosterone from the retro-orbital sinus of grass rats on the first day of DTH sensitization. Blood samples were centrifuged at 4°C for 30 min at 3.3 × *g*, and plasma was pulled off and stored in sealable polypropylene microcentrifuge tubes at –80°C until assayed. Total plasma corticosterone concentrations were determined in duplicate using an ICN Diagnostics ^{125}I double-antibody kit (Costa Mesa, CA). The high and low limits of detectability of the assay were 1200 and 3 ng/mL, respectively. The intra-assay coefficient of variation was 11%. All procedures followed the manufacturer guidelines.

Activity Analyses

A separate cohort of grass rats was implanted intraperitoneally (i.p.; under sterile conditions) with telemeters

(PDT-4000; Minimitter, Bend, OR) while under isoflurane anesthesia. Surgical wounds were treated topically with Betadine (Sigma Chemical) to discourage infection, and grass rats were injected (i.p.) with buprenorphine (.1 mg/kg; Sigma Chemical) in sterile saline to alleviate pain during recovery. Following surgery, grass rats were placed in a clean cage, which was positioned on a receiver (Minimitter) connected to a computer. Receivers collated emitted body temperature and movement activity frequencies continuously over 30-min intervals and converted them to raw data based on preprogrammed calibration curves for each transmitter.

Data Analyses

Tissue weight, body mass, and total surviving CFUs were compared between lighting conditions using a one-way analysis of variance (ANOVA). Delayed-type hypersensitivity swelling and anti-KLH were analyzed by repeated-measures ANOVA with lighting condition as the between-subject factor and time as the within-subject factor. Following a significant result on repeated-measures ANOVA, single-time-point comparisons were made. Plasma corticosterone concentrations were analyzed using a one-tailed *t* test based on a priori hypotheses (Keppel & Wickens, 2004). Fourier analysis was used to determine whether locomotor activity was rhythmic and followed 24-h periodicity using Clocklab software from Actimetrics (Wilmette, IL). Grass rats were considered rhythmic when the highest peak occurred at ~1 cycle/d with an absolute power of at least .005 mV/Hz, as previously described (Kriegsfeld et al., 2008). Fast fourier transform power values for .083 cycles/d were compared between lighting conditions by one-way ANOVA. Percentage of daytime activity and total daily activity were also analyzed by one-way ANOVA. Nonlinear regression analysis was used in GraphPad Prism software (v. 4; La Jolla, CA). In all cases, differences between group means and correlation coefficients were considered statistically significant if $p \leq .05$.

RESULTS

Reproductive and Somatic Measures

There were no differences in body, reproductive tissue, adrenal, spleen, or thymus mass between groups ($p > .05$; Table 1).

Circadian Activity Pattern

There were no differences between grass rats housed in dLAN and those in LD, with respect to circadian pattern in activity, total daily activity, or percentage of activity occurring during the light phase ($p > .05$; Figure 1).

Plasma Corticosterone Concentrations

Samples for corticosterone analysis were collected directly prior to DTH sensitization. Plasma corticosterone concentrations were elevated among grass grass rats exposed to dLAN as compared to those exposed to dark nights ($t_{16} = 1.815$, $p < .05$; Figure 2A).

DTH Swelling Responses

One dLAN grass rat did not develop a swelling response and was excluded from comparisons. The remaining grass rats all exhibited robust swelling of the right pinna over the measurement period ($F_{5,75} = 19.58$, $p < .05$; Figure 2B). There was a main effect of lighting condition, such that grass rats exposed to dLAN had significantly elevated swelling of the right pinna as compared to conspecifics housed with dark nights ($F_{5,75} = 4.30$, $p < .05$). dLAN grass rats had greater swelling on days 2 and 3 post challenge than grass rats exposed to dark nights ($F_{1,15} = 5.62$ and 5.19, respectively, $p < .05$). Furthermore, there was a positive association between plasma corticosterone concentrations and pinna swelling on days 2, 3, and 5 post challenge ($r^2 = .327$, .219, .391, $p \leq .05$; day 2 shown in Figure 2C).

KLH Antibody Production

Two grass rats, one per group, produced no antibody in response to the KLH injection and were excluded from the analyses. Grass rats significantly elevated antibody production following KLH injection ($F_{3,42} = 105.60$, $p < .05$). There was an interaction between light condition and time ($F_{3,42} = 5.21$, $p < .05$), such that grass rats exposed to dLAN increased anti-KLH IgG production 10 and 15 d following injection ($F_{1,14} = 13.11$, 7.67, $p < .05$; Figure 3A).

Bacteria Colony Killing

Grass rats housed in dLAN had decreased percentage of surviving CFUs compared to grass rats housed in standard LD conditions ($F_{1,16} = 8.155$, $p < .05$; Figure 3B).

TABLE 1. Mean body mass (g) and tissue masses (mg) in LD and dLAN grass rats.

	LD	dLAN
Body mass (g)	93.02 ± 6.47	93.55 ± 6.66
Epididymides (mg)	447 ± 19	474 ± 29
Epididymal fat (mg)	2108 ± 315	2230 ± 370
Testes (mg)	1779 ± 67	1713 ± 48
Adrenal (mg)	66 ± 8	62 ± 6
Thymus (mg)	112 ± 20	110 ± 21
Spleen (mg)	142 ± 9	148 ± 12

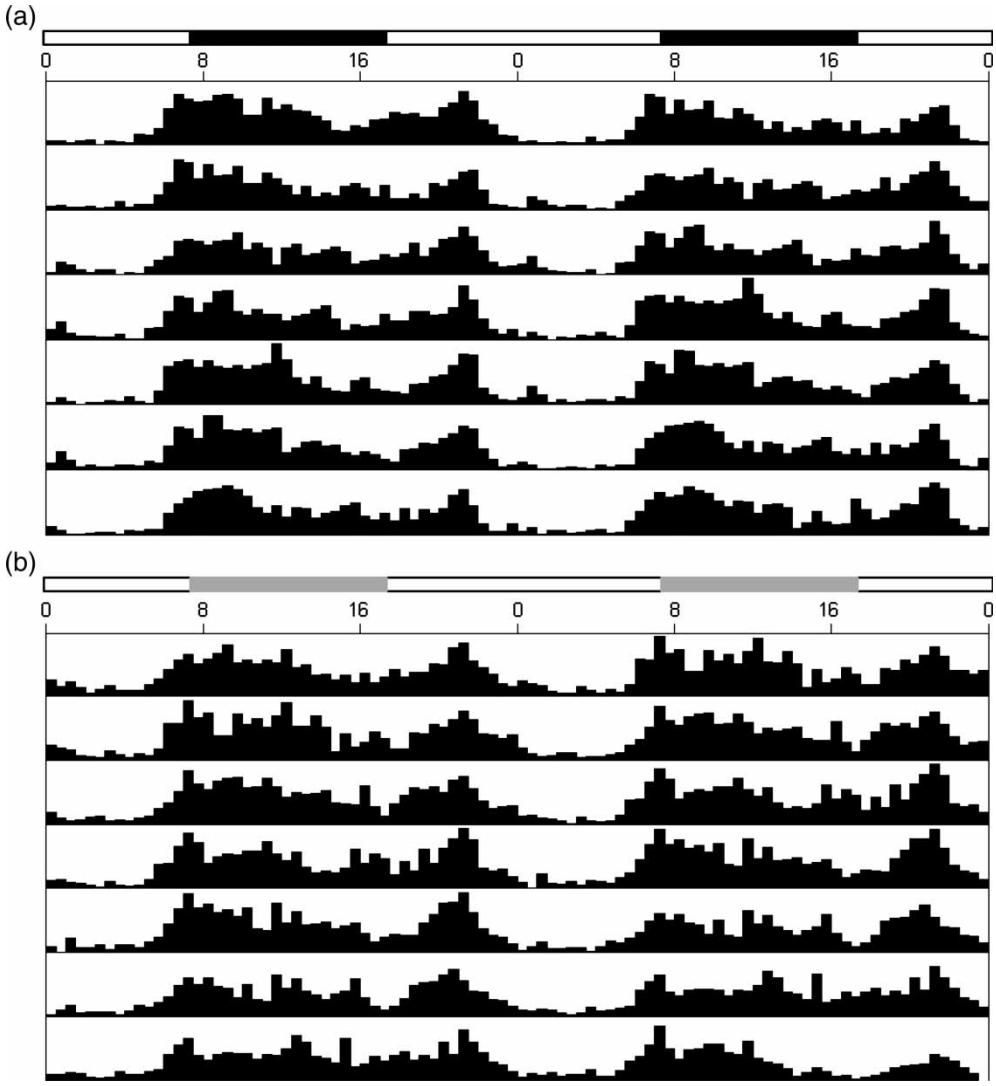

FIGURE 1. No differences were observed in homecage locomotor activity between rats exposed to dim light at night (dLAN) or dark nights. Average actographs from (A) rats housed in standard lighting conditions and (B) rats exposed to dim light at night.

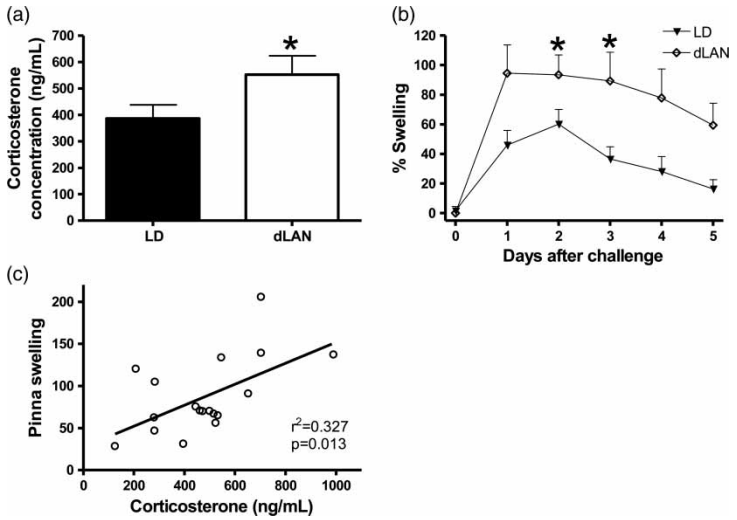

FIGURE 2. Grass rats exposed to dim light at night (dLAN) had (A) elevated plasma corticosterone concentrations as compared with rats under dark nights. (B) dLAN rats had increased pinna swelling 2 and 3 d following challenge with the antigen 2,4-dinitro-1-fluorobenzene. (C) There was an association between peak pinna swelling and corticosterone concentrations 2 d after challenge. Data are presented as mean ± SEM. *$p \leq .05$.

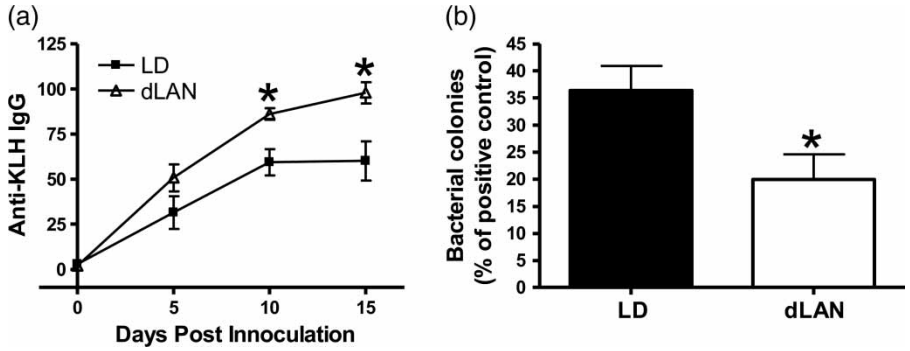

FIGURE 3. Rats exposed to dim light at night (dLAN) demonstrated enhanced humoral immune function and plasma bactericidal capacity. (A) Anti-KLH IgG responses were elevated in dLAN rats 10 and 15 d following inoculation. (B) Plasma obtained from dLAN grass rats showed fewer surviving bacterial colonies. Data are presented as mean ± SEM. *$p < .05$.

DISCUSSION

LAN influenced immune function in male Nile grass rats. Rats exposed to dLAN had elevated bacteriacidal capacity and humoral and cell-mediated immune responses. Increased immune activity occurred independently of overt changes in circadian locomotor activity. dLAN-exposed grass rats increased plasma corticosterone concentrations during the active phase after 3 wks in lighting conditions, which may have affected immunological measures. These results contrast with previously reported results in nocturnal rodents undergoing similar experimental nighttime light exposure. Our results suggest that male and female, as well as diurnal and nocturnal, rodents may respond differently to the effects of nighttime light exposure.

Rats exposed to dLAN increased pinna swelling compared to LD rats in response to the antigen DNFB. DTH is a measure of cell-mediated immunity that demonstrates primary somatic immune response to an invading pathogen. Pinna swelling is caused by increased infiltration of macrophages and lymphocytes into the epidermis and dermis (Vadas et al., 1975) and has previously been positively correlated to the intensity of the immune reaction (Phanuphak et al., 1974). Functionally, elevated swelling in DTH testing is indicative of increased resistance to viruses, bacteria, and fungi (Bilbo et al., 2002).

Increased pinna swelling in dLAN-exposed grass rats contrasts with previously reported results in which swelling was suppressed in LAN-exposed Siberian hamsters (Bedrosian et al., 2011b). DTH responses may vary over the course of the day. In the previous study, Siberian hamsters underwent DTH testing during the light phase when they are generally inactive. In the present study, DTH testing also occurred during the light phase; however, grass rats are diurnal and active at this time. Other factors that vary in a circadian pattern, such as immune cells and hormones, may contribute to the equivocal T cell–mediated results (Bollinger et al., 2010). For example, exposure to dLAN elevates glucocorticoid concentrations in grass rats but not Siberian hamsters (Bedrosian et al., 2011a). Furthermore, glucocorticoid concentration can alter diurnal rhythms in T cell–mediated inflammatory responses, an effect which may be partially mediated by melatonin. Adrenalectomy abolishes the diurnal rhythm in Bacille Calmette-Guérin (BCG) inflammation; however, the rhythm can be recovered with exogenous administration of melatonin (Lopes et al., 2001). In the previous study in Siberian hamsters (Bedrosian et al., 2011b), DTH was also assessed in female Siberian hamsters as compared to male grass rats in the current experiments. Although female Siberian hamsters were ovariectomized, varying levels of sex steroids may have contributed to the divergent results (Bedrosian et al., 2011b). Overall, assessing DTH responses during the active phase in this study is more ecologically relevant, because it is more likely that grass rats would encounter a pathogen while awake and interacting with the external environment.

Exposure to LAN suppresses melatonin production in both rodents and humans (Brainard et al., 1982, 1985; Dauchy et al., 2010). Even very low levels of light exposure can alter melatonin concentrations in rodents (Evans et al., 2007). Although we did not measure melatonin concentrations in this study, it is likely that they were decreased with LAN exposure. It is unlikely, however, that changes in DTH response reflect suppression of melatonin among dLAN rats. Melatonin is positively associated with DTH responses in diurnal and nocturnal rodent species (Drazen & Nelson, 2001; Haldar & Singh, 2001). Glucocorticoids both increase and suppress DTH responses, depending on the type and duration of the stressor (Dhabhar, 2002; Dhabhar & McEwen, 1999). Typically, acute stress increases DTH reactions, whereas chronic stress suppresses DTH reactions, indicating that changes in DTH are in some cases related to altered glucocorticoid concentrations (Dhabhar & McEwen, 1999). During acute stress, blood leukocytes redistribute to the skin, mucosal linings, lung, liver, and lymph nodes—key areas in preventing breaching of immune defenses. In this study, the positive association between corticosterone concentrations and

pinna swelling suggests that the two may be related. It is possible that a long-term stressor, such as LAN, induces a state of functional glucocorticoid resistance. Previous work has demonstrated that psychosocial stressors can cause splenic macrophages to become resistant to the suppressive effects of glucocorticoid hormones (Avitsur et al., 2001, 2002; Bailey et al., 2004). Alternatively, the elevation in glucocorticoids among dLAN rats may be sufficiently low to exert an atypical effect on DTH swelling. Glucocorticoids exert a U-shaped influence on multiple factors; for example, basal or low stress levels of corticosterone enhance glucose utilization, hippocampal synaptic excitability, hippocampal-dependent learning, and cerebral perfusion rate, whereas higher physiological levels of corticosterone exert opposite effects (Sapolsky, 2004). Moreover, melatonin and glucocorticoids interact in modulating immunological processes. Acute and chronic stress increases plasma melatonin concentrations in rodents (Couto-Moraes et al., 2009; Dagnino-Subiabre et al., 2006). This may be a compensatory mechanism, as melatonin protects against some effects of chronic stress (Brotto et al., 2001). This interaction has important implications for this study, because it suggests that glucocorticoids may increase melatonin concentrations partially compensating for the light-induced suppression of melatonin.

Rats exposed to dLAN enhanced antibody production following injection with KLH. Anti-KLH production is a general indicator of B-cell activity. Previous studies have reported no differences in anti-KLH production or enhanced production in melatonin-treated rodents (Demas et al., 1997; Drazen & Nelson, 2001). Furthermore, primary and secondary antibody production is decreased in mice treated with propranolol (Maestroni et al., 1986). Thus, differences in antibody production between dLAN and LD rats may be independent of putative changes in melatonin. Elevated concentrations of glucocorticoids following social defeat are associated with enhanced lymphocyte release of IFNγ and IL6. However, changes in anti-KLH production are not apparent (Merlot et al., 2004). Furthermore, in another model of social defeat, elevated glucocorticoid concentrations were associated with impairment in antiviral immunological memory (de Groot et al., 2002). Again, this indicates that changes in anti-KLH production may occur independently of changes in corticosteroids. Alternatively, LAN may be an atypical stressor, impacting the glucocorticoid system in a different manner than other chronic stressors.

Bactericidal capacity was enhanced in dLAN rats as compared to those housed in standard lighting conditions. Bacteria killing is a low-cost, nonspecific immune response predominately mediated by plasma proteins (Martin et al., 2007). Plasma bactericidal capacity increases with immune challenge and represents an enhanced ability to clear a bacterial infection (Weinrauch et al., 1998). Elevated glucocorticoid concentrations have been associated with enhanced bactericidal capacity in spleen cells from mice that underwent social disruption stress (Bailey et al., 2007). Melatonin, however, generally enhances bactericidal capacity (Terron et al., 2009).

Overall, nighttime light exposure increased immunocompetence in grass rats. LAN may exert its effects through changes in the hypothalamic-pituitary adrenal axis, as corticosteroid concentrations were elevated among dLAN rats. It is widely accepted that stress affects immune responses, with chronic stress generally exerting an immunosuppressive effect. The role of glucocorticoids in immunological processes are complex, however (Sorrells et al., 2009); depending on the type of stress, glucocorticoids can have opposite effects. LAN may also disrupt circadian processes leading to changes in immune function. Although gross changes in locomotor activity were not apparent, disruption of the circadian system at the molecular level may have occurred. Previous work has indicated disruption of clock function can lead to exacerbated immune activity (Arjona & Sarkar, 2006; Castanon-Cervantes et al., 2010; Hashiramoto et al., 2010). Melatonin can also be directly produced by immune cells (Pontes et al., 2006). Importantly, this study did not evaluate all aspects of immune function; it is possible that other arms of the immune system could be differentially affected by nighttime light exposure (Martin et al., 2007).

Enhanced immune responses are not always favorable. Immunological processes are energetically costly and a delegation of energy to immune responses in unnecessary situations can reduce fitness (Martin et al., 2008). Moreover, enhancement of the immune response in cases such as allergic asthma is detrimental (Wills-Karp, 1999). Because of the contrasting results obtained in this study and another study conducted on a nocturnal rodent species, further research on the effects of LAN on immune function is warranted. Nighttime light exposure is currently experienced by over 99% of the population of the United States and Europe. Light pollution has significant ecological consequences for animals living in urban and suburban areas and may contribute to species loss (Navara & Nelson, 2007). It is important to understand the physiological implications of this exposure in order to work toward preventing ecologically related complications.

ACKNOWLEDGMENTS

We thank Laura Smale for providing the grass rats and for consultation on the study. We also thank Zachary M. Weil and James C. Walton for assistance with this study and Sallion Wolfe for excellent animal care. This research was supported by NSF grant IOS 04-16897 and by United States–Israel Binational Science Foundation grant 2005-337.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

Arjona A, Sarkar DK. (2006). The circadian gene mPer2 regulates the daily rhythm of IFN-gamma. *J. Interferon Cytokine Res*. 26:645–649.

Avitsur R, Stark JL, Sheridan JF. (2001). Social stress induces glucocorticoid resistance in subordinate animals. *Horm. Behav*. 39:247–257.

Avitsur R, Stark JL, Dhabhar FS, Padgett DA, Sheridan JF. (2002). Social disruption-induced glucocorticoid resistance: kinetics and site specificity. *J. Neuroimmunol.* 124:54–61.

Bailey MT, Avitsur R, Engler H, Padgett DA, Sheridan JF. (2004). Physical defeat reduces the sensitivity of murine splenocytes to the suppressive effects of corticosterone. *Brain Behav. Immun.* 18:416–424.

Bailey MT, Engler H, Powell ND, Padgett DA, Sheridan JF. (2007). Repeated social defeat increases the bactericidal activity of splenic macrophages through a Toll-like receptor-dependent pathway. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 293: R1180–R1190.

Bedrosian TA, Fonken LK, Walton JC, Haim A, Nelson RJ (2011a). Dim light at night provokes depression-like behaviors and reduces CA1 dendritic spine density in female hamsters. *Psychoneuroendocrinology*. 36:1062–1069.

Bedrosian TA, Fonken LK, Walton JC, Nelson RJ. (2011b). Chronic exposure to dim light at night suppresses immune responses in Siberian hamsters. *Biol. Lett.* 7:468–471.

Bilbo SD, Dhabhar FS, Viswanathan K, Saul A, Yellon SM, Nelson RJ. (2002). Short day lengths augment stress-induced leukocyte trafficking and stress-induced enhancement of skin immune function. *Proc. Natl. Acad. Sci. U. S. A.* 99:4067–4072.

Blask DE. (2009). Melatonin, sleep disturbance and cancer risk. *Sleep Med. Rev*. 13:257–264.

Bollinger T, Bollinger A, Naujoks J, Lange T, Solbach W. (2010). The influence of regulatory T cells and diurnal hormone rhythms on T helper cell activity. *Immunology*. 131:488–500.

Borugian MJ, Gallagher RP, Friesen MC, Switzer TF, Aronson KJ. (2005). Twenty-four-hour light exposure and melatonin levels among shift workers. *J. Occup. Environ. Med.* 47:1268–1275.

Brainard GC, Richardson BA, Petterborg LJ, Reiter RJ. (1982). The effect of different light intensities on pineal melatonin content. *Brain Res.* 233:75–81.

Brainard GC, Lewy AJ, Menaker M, Fredrickson RH, Miller LS, Weleber RG, Cassone V, Hudson D. (1985). Effect of light wavelength on the suppression of nocturnal plasma melatonin in normal volunteers. *Ann. N. Y. Acad. Sci.* 453:376–378.

Brotto LA, Gorzalka BB, LaMarre AK. (2001). Melatonin protects against the effects of chronic stress on sexual behaviour in male rats. *Neuroreport*. 12:3465–3469.

Castanon-Cervantes O, Wu M, Ehlen JC, Paul K, Gamble KL, Johnson RL, Besing RC, Menaker M, Gewirtz AT, Davidson AJ. (2010). Dysregulation of inflammatory responses by chronic circadian disruption. *J. Immunol.* 185:5796–5805.

Cecon E, Fernandes PA, Pinato L, Ferreira ZS, Markus RP. (2010). Daily variation of constitutively activated nuclear factor kappa B (NFKB) in rat pineal gland. *Chronobiol. Int.* 27:52–67.

Chen Z, Gardi J, Kushikata T, Fang J, Krueger JM. (1999). Nuclear factor-kappaB-like activity increases in murine cerebral cortex after sleep deprivation. *Am. J. Physiol.* 276:R1812–R1818.

Claustrat B, Valatx JL, Harthe C, Brun J. (2008). Effect of constant light on prolactin and corticosterone rhythms evaluated using a noninvasive urine sampling protocol in the rat. *Horm. Metab. Res.* 40:398–403.

Couto-Moraes R, Palermo-Neto J, Markus RP. (2009). The immune-pineal axis: stress as a modulator of pineal gland function. *Ann. N. Y. Acad. Sci.* 1153:193–202.

da Silveira Cruz-Machado S, Carvalho-Sousa CE, Tamura EK, Pinato L, Cecon E, Fernandes PA, de Avellar MC, Ferreira ZS, Markus RP. (2010). TLR4 and CD14 receptors expressed in rat pineal gland trigger NFKB pathway. *J. Pineal Res.* 49:183–192.

Dagnino-Subiabre A, Orellana JA, Carmona-Fontaine C, Montiel J, Diaz-Veliz G, Seron-Ferre M, Wyneken U, Concha ML, Aboitiz F. (2006). Chronic stress decreases the expression of sympathetic markers in the pineal gland and increases plasma melatonin concentration in rats. *J. Neurochem.* 97:1279–1287.

Dauchy RT, Dauchy EM, Tirrell RP, Hill CR, Davidson LK, Greene MW, Tirrell PC, Wu J, Sauer LA, Blask DE. (2010). Dark-phase light contamination disrupts circadian rhythms in plasma measures of endocrine physiology and metabolism in rats. *Comp. Med.* 60:348–356.

Davis S, Mirick DK. (2006). Circadian disruption, shift work and the risk of cancer: a summary of the evidence and studies in Seattle. *Cancer Causes Control*. 17:539–545.

de Groot J, Boersma WJ, Scholten JW, Koolhaas JM. (2002). Social stress in male mice impairs long-term antiviral immunity selectively in wounded subjects. *Physiol. Behav.* 75:277–285.

Deboer T, Detari L, Meijer JH. (2007). Long term effects of sleep deprivation on the mammalian circadian pacemaker. *Sleep*. 30:257–262.

Demas GE, Chefer V, Talan MI, Nelson RJ. (1997). Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *Am. J. Physiol.* 273:R1631–R1637.

Dhabhar FS. (2002). Stress-induced augmentation of immune function—the role of stress hormones, leukocyte trafficking, and cytokines. *Brain Behav. Immun.* 16:785–798.

Dhabhar FS, McEwen BS. (1999). Enhancing versus suppressive effects of stress hormones on skin immune function. *Proc. Natl. Acad. Sci. U. S. A.* 96:1059–1064.

Drazen DL, Nelson RJ. (2001). Melatonin receptor subtype MT2 (Mel 1b) and not mt1 (Mel 1a) is associated with melatonin-induced enhancement of cell-mediated and humoral immunity. *Neuroendocrinology*. 74:178–184.

Dumont M, Beaulieu C. (2007). Light exposure in the natural environment: relevance to mood and sleep disorders. *Sleep Med.* 8:557–565.

Evans JA, Elliott JA, Gorman MR. (2007). Circadian effects of light no brighter than moonlight. *J. Biol. Rhythms*. 22:356–367.

Fiske VM. (1941). Effect of light on sexual maturation, estrous cycles, and anterior pituitary of the rat. *Endocrinology*. 29:187–196.

Fonken LK, Nelson RJ. (2011). Illuminating the deleterious effects of light at night. *F1000 Med. Rep.* 3:18.

Fonken LK, Finy MS, Walton JC, Weil ZM, Workman JL, Ross J, Nelson RJ. (2009). Influence of light at night on murine anxiety- and depressive-like responses. *Behav. Brain Res.* 205:349–354.

Fonken LK, Workman JL, Walton JC, Weil ZM, Morris JS, Haim A, Nelson RJ. (2010). Light at night increases body mass by shifting the time of food intake. *Proc. Natl. Acad. Sci. U. S. A.* 107:18664–18669.

Gilad E, Wong HR, Zingarelli B, Virag L, O'Connor M, Salzman AL, Szabo C. (1998). Melatonin inhibits expression of the inducible isoform of nitric oxide synthase in murine macrophages: role of inhibition of NFkappaB activation. *FASEB J.* 12:685–693.

Ha M, Park J. (2005). Shiftwork and metabolic risk factors of cardiovascular disease. *J. Occup. Health*. 47:89–95.

Haldar C, Singh R. (2001). Pineal modulation of thymus and immune function in a seasonally breeding tropical rodent, *Funambulus pennanti*. *J. Exp. Zool.* 289:90–98.

Hashiramoto A, Yamane T, Tsumiyama K, Yoshida K, Komai K, Yamada H, Yamazaki F, Doi M, Okamura H, Shiozawa S. (2010). Mammalian clock gene Cryptochrome regulates arthritis via proinflammatory cytokine TNF-alpha. *J. Immunol.* 184:1560–1565.

Hut RA, Beersma DG. (2011). Evolution of time-keeping mechanisms: early emergence and adaptation to photoperiod. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 366:2141–2154.

Kanda N, Watanabe S. (2005). Regulatory roles of sex hormones in cutaneous biology and immunology. *J. Dermatol. Sci.* 38:1–7.

Keppel G, Wickens T. (2004). *Design and analysis: a researcher's handbook*. 4th ed. Upper Saddle River, NJ: Prentice Hall, 73–75.

Klein SL, Nelson RJ. (1999). Social interactions unmask sex differences in humoral immunity in voles. *Anim. Behav.* 57:603–610.

Kloog I, Portnov BA, Rennert HS, Haim A. (2011). Does the modern urbanized sleeping habitat pose a breast cancer risk? *Chronobiol. Int.* 28:76–80.

Kohyama J. (2009). A newly proposed disease condition produced by light exposure during night: asynchronization. *Brain Dev.* 31:255–273.

Kondratov RV, Kondratova AA, Gorbacheva VY, Vykhovanets OV, Antoch MP. (2006). Early aging and age-related pathologies in mice deficient in BMAL1, the core componentof the circadian clock. *Genes Dev.* 20:1868–1873.

Kriegsfeld LJ, Mei DF, Yan L, Witkovsky P, Lesauter J, Hamada T, Silver R. (2008). Targeted mutation of the calbindin D28K gene disrupts circadian rhythmicity and entrainment. *Eur. J. Neurosci.* 27:2907–2921.

Lopes C, Mariano M, Markus RP. (2001). Interaction between the adrenal and the pineal gland in chronic experimental inflammation induced by BCG in mice. *Inflamm. Res.* 50:6–11.

Lotufo CM, Lopes C, Dubocovich ML, Farsky SH, Markus RP. (2001). Melatonin and *N*-acetylserotonin inhibit leukocyte rolling and adhesion to rat microcirculation. *Eur. J. Pharmacol.* 430:351–357.

Lundkvist GB, Robertson B, Mhlanga JD, Rottenberg ME, Kristensson K. (1998). Expression of an oscillating interferon-gamma receptor in the suprachiasmatic nuclei. *Neuroreport.* 9:1059–1063.

Maestroni GJ, Conti A, Pierpaoli W. (1986). Role of the pineal gland in immunity. Circadian synthesis and release of melatonin modulates the antibody response and antagonizes the immunosuppressive effect of corticosterone. *J. Neuroimmunol.* 13:19–30.

Markus RP, Ferreira ZS, Fernandes PA, Cecon E. (2007). The immune-pineal axis: a shuttle between endocrine and paracrine melatonin sources. *Neuroimmunomodulation*. 14:126–133.

Martin LB 2nd, Weil ZM, Nelson RJ. (2007). Immune defense and reproductive pace of life in *Peromyscus* mice. *Ecology*. 88:2516–2528.

Martin LB, Weil ZM, Nelson RJ. (2008). Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 363:321–339

Martinez-Nicolas A, Ortiz-Tudela, Madrid JA, Rol MA. (2011). Crosstalk between environmental light and internal time in humans. *Chronobiol. Int.* 28:617–629.

Merlot E, Moze E, Dantzer R, Neveu PJ. (2004). Cytokine production by spleen cells after social defeat in mice: activation of T cells and reduced inhibition by glucocorticoids. *Stress*. 7:55–61.

Navara KJ, Nelson RJ. (2007). The dark side of light at night: physiological, epidemiological, and ecological consequences. *J. Pineal Res.* 43:215–224.

Negi G, Kumar A, Sharma SS. (2011). Melatonin modulates neuroinflammation and oxidative stress in experimental diabetic neuropathy: effects on NF-kappaB and Nrf2 cascades. *J. Pineal Res.* 50:124–131.

Parry BL, Meliska CJ, Sorenson DL, Lopez A, Martinez LF, Hauger RL, Elliot JA. (2010). Increased sensitivity to light-induced melatonin suppression in premenstrual dysphoric disorder. *Chronobiol. Int.* 27:1438–1453.

Persengiev S, Kanchev L, Vezenkova G. (1991). Circadian patterns of melatonin, corticosterone, and progesterone in male rats subjected to chronic stress: effect of constant illumination. *J. Pineal Res.* 11:57–62.

Phanuphak P, Moorhead JW, Claman HN. (1974). Tolerance and contact sensitivity to DNFB in mice. I. In vivo detection by ear swelling and correlation with in vitro cell stimulation. *J. Immunol.* 112:115–123.

Pontes GN, Cardoso EC, Carneiro-Sampaio MM, Markus RP. (2006). Injury switches melatonin production source from endocrine (pineal) to paracrine (phagocytes)—melatonin in human colostrum and colostrum phagocytes. *J. Pineal Res.* 41:136–141.

Portaluppi F, Smolensky MH, Touitou Y. (2010). Ethics and methods for biological rhythm research on animals and human beings. *Chronobiol. Int.* 27:1911–1929.

Puttonen S, Viitasalo K, Härma M. (2011). Effect of shiftwork on systemic markers of inflammation. *Chronobiol. Int.* 28:528–535.

Reiter RJ, Tan DX, Korkmaz A, Ma S. (2011). Obesity and metabolic syndrome: association with chronodisruption, sleep deprivation, and melatonin suppression. *Ann. Med.* doi: 10.3109/07853890.2011.586365

Reppert SM, Weaver DR. (2002). Coordination of circadian timing in mammals. *Nature*. 418:935–941.

Sapolsky RM. (2004). Stress and cognition. Gazzaniga M. *The cognitive neuroscience III*. Cambridge, MA: MIT press, 1031–1042

Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, Kawachi I, Colditz GA. (2001). Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. *J. Natl. Cancer Inst.* 93:1563–1568.

Sorrells SF, Caso JR, Munhoz CD, Sapolsky RM. (2009). The stressed CNS: when glucocorticoids aggravate inflammation. *Neuron*. 64:33–39.

Stevens RG. (2011). Testing the light-at-night (LAN) theory for breast cancer causation. *Chronobiol. Int.* 28:653–656.

Tamura EK, Cecon E, Monteiro AW, Silva CL, Markus RP. (2009). Melatonin inhibits LPS-induced NO production in rat endothelial cells. *J. Pineal Res.* 46:268–274.

Terron MP, Delgado J, Paredes SD, Barriga C, Reiter RJ, Rodriguez AB. (2009). Effect of melatonin and tryptophan on humoral immunity in young and old ringdoves (*Streptopelia risoria*). *Exp. Gerontol.* 44:653–658.

Thomas BB, Oommen MM, Ashadevi O. (2001). Constant light and blinding effects on reproduction of male South Indian gerbils. *J. Exp. Zool.* 289:59–65.

Vadas MA, Miller JF, Gamble J, Whitelaw A. (1975). A radioisotopic method to measure delayed type hypersensitivity in the mouse. I. Studies in sensitized and normal mice. *Int. Arch. Allergy Appl. Immunol.* 49:670–692.

Weil ZM, Martin LB, 2nd, Nelson RJ. (2006). Photoperiod differentially affects immune function and reproduction in collared lemmings (*Dicrostonyx groenlandicus*). *J. Biol. Rhythms*. 21:384–393.

Weinrauch Y, Abad C, Liang NS, Lowry SF, Weiss J. (1998). Mobilization of potent plasma bactericidal activity during systemic bacterial challenge. Role of group IIA phospholipase A2. *J. Clin. Invest.* 102:633–638.

Wills-Karp M. (1999). Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu. Rev. Immunol.* 17:255–281.

Young MR, Matthews JP, Kanabrocki EL, Sothern RB, Roitman-Johnson B, Scheving LE. (1995). Circadian rhythmometry of serum interleukin-2, interleukin-10, tumor necrosis factor-alpha, and granulocyte-macrophage colony-stimulating factor in men. *Chronobiol. Int.* 12:19–27.