RESEARCH ARTICLE

Dim Light at Night Interferes With the Development of the Short-Day Phenotype and Impairs Cell-Mediated Immunity in Siberian Hamsters (*Phodopus sungorus*)

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ABSTRACT

Winter is a challenging time to survive and breed outside of the tropics. Animals use day length (photoperiod) to regulate seasonally appropriate adaptations in anticipation of challenging winter conditions. The net result of these photoperiod-mediated adjustments is enhanced immune function and increased survival. Thus, the ability to discriminate day length information is critical for survival and reproduction in small animals. However, during the past century, urban and suburban development has rapidly expanded and filled the night sky with light from various sources, obscuring crucial light-dark signals, which alters physiological interpretation of day lengths. Furthermore, reduced space, increased proximity to people, and the presence of light at night may act as stressors for small animals. Whereas acute stressors typically enhance immune responses, chronic exposure to stressors often impairs immune responses. Therefore, we hypothesized that the combination of dim light at night and chronic stress interferes with enhanced cell-mediated immunity observed during short days. Siberian hamsters (*Phodopus sungorus*) were assigned to short or long days with dark nights (0 lux) or dim (5 lux) light at night for 10 weeks. Following 2 weeks of chronic restraint (6 hr/day), a model of chronic stress, delayed type hypersensitivity (DTH) responses were assessed. Both dim light at night and restraint reduced the DTH response. Dim light at night during long nights produced an intermediate short day phenotype. These results suggest the constant presence of light at night could negatively affect survival of photoperiodic rodents by disrupting the timing of breeding and immune responses. *J. Exp. Zool. 321A:450–456, 2014.* © 2014 Wiley Periodicals, Inc.

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Abbreviations: DTH, delayed-type hypersensitivity; DNFB, 2,4-dinitro-1-fluorobenzene; LD, long days and dark nights; LDdim, long days and dim light at night; SD, short days and dark nights; SDdim, short days and dim light at night; UR, unrestrained; R, restrained; ZT, Zeitgeber time.

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Small mammals are declining in numbers worldwide (Stuart et al., 2004; Schipper et al., 2008). Factors such as habitat destruction and environmental toxins likely contribute to this decline (Schipper et al., 2008). Another pervasive, yet widely overlooked, factor that might contribute to reduced numbers of small mammals is light at night. Because of increased urbanization and the use of electric lighting, night in many areas is no longer dark. Street lights can produce ~5–60 lux (Gaston et al., 2012), depending on the distance from the light source; whereas moonlight typically is between 0.1 and 0.3 lux (Longcore and Rich, 2004). Melatonin production is responsive to light and can be disrupted by nighttime light exposure (Brainard et al., '82). Because many vertebrates use the duration of nighttime melatonin production to time seasonal breeding, perturbations of this system may disrupt appropriate timing of physiology and behavior. The increase in nighttime light levels can potentially affect the wildlife in urban and suburban areas by obscuring environmental light and dark signals.

The annual cycle of changing day length (photoperiod) provides a reliable environmental cue to determine the time of year (Goldman, 2001). This temporal information is important for timing onset and termination of seasonally appropriate adaptations that promote reproduction and survival (Nelson, 2004). For example, individuals of many species use photoperiod, encoded by the nightly duration of melatonin secretion, to accurately time breeding. Light at night during the winter could alter testosterone concentrations; maintaining high blood testosterone concentrations could result in inappropriate social behaviors, poor physiological adaptation to winter conditions, and out-of-season breeding. Testosterone is increased earlier in spring in birds exposed to light at night than birds exposed to dark nights; however, the effects of light at night on testosterone levels in mammals remain unspecified (Dominoni et al., 2013d). Typically, outside of the tropics, offspring are produced during spring or summer when food is most abundant, and other environmental conditions are optimal for survival. Winter is often a challenging time to breed and survive because of the energetic bottleneck resulting from increased thermoregulatory demands when food availability is scarce (Nelson and Demas, '97). Immune function often varies on a seasonal basis; it is generally decreased during the winter in the wild, but is enhanced in the laboratory during short-day conditions, when all other factors are held constant (Demas and Nelson, '98). Many small animals use day length information to anticipate winter stressors and accordingly redistribute energy among competing reproductive and survival functions. Thus, investment in reproduction (and growth) is curtailed, whereas investment in immune function is bolstered during winter in response to short day lengths.

Melatonin functions as the biological signal for day length or, more precisely, night length. Pineal melatonin synthesis and secretion occurs at night and is inhibited directly by light (Brainard et al., '82). The duration of its release is proportional to night length; consequently, short-day animals experience longer durations of melatonin secretion, than do long-day animals, and use this temporal information to determine the time of the year.

Distinct light and dark signals are thus important for light entrainment of biological clocks that coordinate temporal regulation of physiology and behavior (Fonken et al., 2013). Melatonin secretion is exquisitely responsive to environmental light (Burke et al., 2013). Thus, exposure to light at night can disrupt the long-night signal and potentially provoke a mismatch between environmental conditions and physiological and behavioral adaptations which could lead to breeding at the wrong time of year and compromised immune function.

Immune responses are regulated by changes in day length. Delayed-type hypersensitivity is a reliable method to assess cell-mediated immune function and correlates with increased circulating leukocytes (Bilbo et al., 2002; Prendergast et al., 2013). Hamsters enhance cell-mediated immune responses in short compared to long days and acute stress augments this effect (Bilbo and Nelson, 2003). As noted, short days enhance immune function to increase the chances of survival (Yellon et al., '99; Nelson, 2004); however, light at night impairs cell-mediated immune responses in Siberian hamsters (Bedrosian et al., 2011), Japanese quail (Moore and Siopes, 2000), cockerels, (Kirby and Froman, '91), and rats (Oishi et al., 2006).

Distinct light-dark signals are required for photoperiodic responses including enhanced immune responses. Dim light at night interfering with development of the short-day phenotype could lead to inappropriate breeding times, as well as impaired immune function. Additionally, chronic stressors compromise immune function. Therefore, we hypothesize that dim light at night and chronic stress could combine to blunt short day enhancement of cell-mediated immunity.

MATERIALS AND METHODS

Animals

Sixty-five adult (>8 weeks) male Siberian hamsters (*Phodopus sungorus*) born in our breeding colony at The Ohio State University were used ($N = 8$/group except SD restrained, $N = 9$). Hamsters were group housed in propylene cages (dimensions: 30 cm × 15 cm × 14 cm) at an ambient temperature of 22 ± 2°C, relative humidity of 50% ± 10%, and provided with food (Harlan Teklad 8640, Indianapolis, IN, USA) and filtered tap water ad libitum. Hamsters were maintained throughout the experiment on (1) long days (LD; 16 hr light/8 hr dark; 150 lux/0 lux), (2) short days (SD; 8 hr light/16 hr dark (150 lux/0 lux), (3) long days and dimly lit nights (LDdim; 16 hr light/8 hr dim light; 150 lux/5 lux), or (4) short days and dimly lit nights (SDdim; 8 hr light/16 hr dim light; 150 lux/5 lux). Hamsters were in light treatment for eight weeks before the beginning of restraint stress. On the first day of exposure to chronic restraint stress all hamsters were moved to individual housing for the remainder of the experiment.

Restraint

Following 8 weeks in light treatment hamsters were placed in ventilated Plexiglas restraint tubes (6 hr/day for 2 weeks) during the daily light phase to provoke a stress response. Unrestrained hamsters were kept in their home cage without access to food and water for the same duration of the restraint each day. Blood was collected from anesthetized hamsters via the retro-orbital sinus at the end of the final restraint period. Hamsters were then injected with 0.1 mL of sterile saline to prevent dehydration.

Delayed-Type Hypersensitivity

Delayed-type hypersensitivity (DTH) was induced by sensitization to, and later challenge with, the antigen 2,4-dinitro-1-fluorobenzene (DNFB) (Sigma–Aldrich, St. Louis, MO, USA) as described previously (Bilbo and Nelson, 2003). Hamsters were sensitized with 25 μL of DNFB (0.5% in 4:1 acetone to olive oil vehicle) applied to the shaved dorsum on two consecutive days (day 7 and 8 of restraint, 2 hr before restraint at *Zeitgeber* Time (ZT) 4). ZT is a convention in circadian biology where ZT 12 (for nocturnal animals) is defined as the time of lights off (1500 Eastern Standard Time). Following the last restraint session (seven days after the start of sensitization) hamsters were bled, baseline pinna measurements recorded, and then challenged on the right pinna with 20 μL of DNFB 0.2% in acetone-olive oil vehicle. The left pinna was treated with vehicle. Following challenge, measurements of pinnae thickness were recorded every 24 hr for 6 days. Pinnae measurements were made at the same time every day, between ZT 9-11 and hamsters were individually brought into the room to minimize potential stress responses.

Body Mass and Pelage

Hamsters were weighed at the end of restraint treatment prior to euthanasia. Pelage was scored at the time of euthanasia on a 1–5 scale with 5 = white, winter pelage and 1 = dark, summer pelage. Pelage was scored via photograph by a condition-blind observer.

Tissue Collection

Hamsters were anesthetized with isoflurane anesthesia and blood was collected via the retro-orbital sinus between ZT 6–9. Hamsters were then injected with an overdose of euthasol. Once the hamster was nonresponsive, spleens, testes, epididymides, inguinal fat pads, and seminal vesicles were removed and weighed.

Testosterone Concentrations

Terminal blood samples were collected via the retro-orbital sinus for radioimmunoassay of testosterone concentrations. Samples incubated at room temperature for ~30 min and then centrifuged at 4,300 rotations per min for 30 min at 4°C, then plasma aliquots were stored at −80°C until assayed. Total plasma testosterone concentrations were determined in duplicate using an ^{125}I antibody kit (MP Biomedicals, Solon, OH, USA), ^{125}I antibody kits have been validated previously for use in Siberian hamsters (Weil et al., 2007). The high and low limits of detectability of the assay were 0.1 and 10 ng/mL, respectively. The intra-assay coefficient of variation was <10%, as calculated from a low (0.1 ng/mL), medium (1 ng/mL), and high control (10 ng/mL) provided in the kit. All procedures were performed in accordance with the manufacturer's instructions.

Statistical Analyses

Main effects of day length (long-day, short-day), nighttime lighting (dark, dim light at night), restraint (unrestrained and restrained) and interactions among the three variables were assessed. A $2 \times 2 \times 2$ repeated measures ANOVA was used to compare percent change in pinnae thickness after DNFB challenge. Final pelage was analyzed with a $2 \times 2 \times 2$ univariate ANOVA. Body mass at the end of restraint and testosterone concentrations had unequal variance as determined by Levene's test of equality of error variances for both body mass (degrees of freedom 7.55) and testosterone (degrees of freedom 7.54); therefore, a Kruskal–Wallis test was used to determine differences and followed up by Mann–Whitney *U* post hoc tests. Tissue masses: spleen, testes, epididymides, inguinal fat pads, and seminal vesicles were analyzed with a $2 \times 2 \times 2$ multivariate ANOVA with final body mass as a covariate. Statistics were performed using SPSS 19 for Windows (IBM, Armonk, New York, USA). Outliers were determined by *Z* score (±2 standard deviations (SD)) were removed as listed in the results section. Mean differences were considered statistically significant when P was ≤0.05.

RESULTS

Delayed-Type Hypersensitivity (DTH)

Restraint impaired cell-mediated immunity overall ($F_{6,294} = 3.234$, $P < 0.01$, Fig. 1A and B). Overall, restrained hamsters in long days and dark nights had reduced inflammation compared to hamsters exposed to short days and dark nights for both restrained and unrestrained hamsters ($P < 0.05$, Fig. 1B). Overall, exposure to dim light at night impaired cell-mediated immunity as measured by pinnae swelling ($F_{6,294} = 2.391$, $P < 0.05$, Fig. 1D and E). Dim light at night exposure in unrestrained short day hamsters reduced inflammation on Days 1, 2, and 3 compared to unrestrained short day hamsters with dark nights ($P < 0.05$, Fig. 1C). On day 1 dim light at night also reduced inflammation in restrained hamsters exposed to short days and dim light at night compared to restrained short day hamsters with dark nights ($P < 0.05$, Fig. 1F). As previously reported, short days increased DTH, pinnae thickness on Day 1, 2, and 3 was higher in restrained hamsters in short days and dark nights compared to restrained hamsters in long-days and dark nights ($P < 0.05$, Fig. 1B) (Bilbo et al., 2002). Dim light at night blocked the short day-enhancement of cell-mediated immunity ($F_{6,294} = 4.509$, $P < 0.01$, Fig. 1C and F). Pinnae thickness on Day 1 and 2 was increased in unrestrained short-days and dark nights compared to

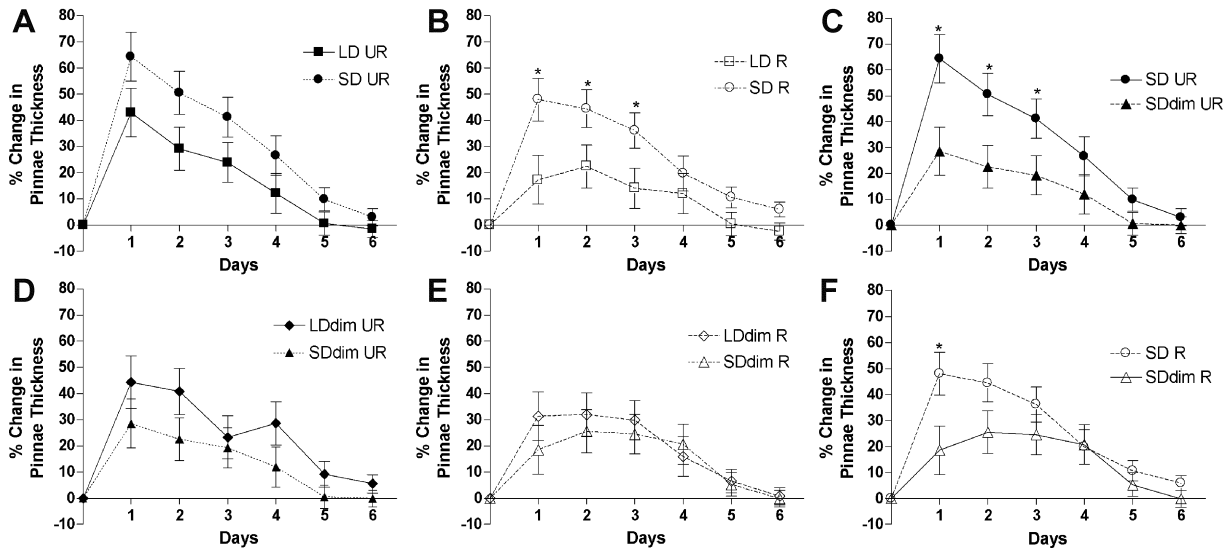

Figure 1. Overall, dim light at night and restraint impaired delayed-type hypersensitivity (DTH) responses; this was most pronounced in short day Siberian hamsters (*Phodopus sungorus*). Mean (±standard error of the mean (SEM)) of percentage change in pinnae thickness the days following challenge with antigen DNFB. LD, long days and dark nights; SD, short days and dark nights; LDdim, long days and dim light at night; SDdim, short days and dim light at night; UR, unrestrained; R, restrained. There were no differences in pinnae swelling on individual days between UR LD or SD hamsters (A). LD R impaired cell-mediated immunity compared to SD R the first three days following challenge, * indicate significance at $P < 0.05$ (B). SDdim UR impaired cell-mediated immunity compared to SD UR hamsters the first three days following challenge, * indicate significance at $P < 0.05$ (C). LDdim and SDdim unrestrained hamsters had similar pinnae swelling across individual days (D). Restrained LD and SD hamsters had similar pinnae swelling across individual days (E). SDdim R impaired cell-mediated immunity compared to SD R hamsters the first day following challenge with DNFB, * indicate significance at $P < 0.05$ (F).

hamsters in short-days and dim light at night ($P < 0.05$, Fig. 1). Five hamsters did not exhibit any inflammation (three from long day/dim nights and one from long days and one from short days) and were removed from analyses; an additional three hamsters were outliers (two from short day/dim night, one from long day conditions) based on Z score and were removed from analyses.

Tissue Masses and Pelage

Short days decreased paired epididymides mass ($F_{1,\ 53} = 18.407$, $P < 0.01$, Fig. 2A), testes mass ($F_{1,\ 53} = 38.477$, $P < 0.01$, Fig. 2B), and inguinal fat pad mass ($F_{1,\ 53} = 23.462$, $P < 0.01$, Fig. 2C) compared to long days. Short days increased spleen mass ($F_{1,\ 53} = 9.013$, $P < 0.01$, Fig. 2D) compared to long days. Dim light at night increased paired epididymides mass ($F_{1,\ 53} = 8.619$, $P < 0.01$, Fig. 2A) and testes mass ($F_{1,\ 53} = 12.712$, $P < 0.01$, Fig. 2B) compared to dark nights. Inguinal fat pad mass and spleen mass were not altered by nighttime lighting ($P > 0.05$, Fig. 2C and D). Restraint decreased paired testes mass ($F_{1,\ 53} = 10.716$, $P < 0.01$, Fig. 2B) and fat pad mass ($F_{1,\ 53} = 11.276$, $P < 0.01$, Fig. 2C) compared to unrestrained hamsters. Restraint did not alter any other tissue masses ($P > 0.05$, Fig. 2). Dim light at night interacted with day length such that hamsters exposed to short days and dim light at night exhibited larger paired testes mass ($F_{1,\ 53} = 6.899$, $P < 0.05$, Fig. 2B) than short day hamsters in dark nights. One hamster in the short day restrained group and one in the long day unrestrained group had outlying paired testes mass based on Z score (±2 SD) and were removed from all tissue mass analyses.

Final pelage was lighter in short days than long days ($F_{1,\ 57} = 58.558$, $P < 0.05$, Fig. 2E). Dim light at night darkened pelage score compared to dark nights ($F_{1,\ 57} = 4.394$, $P < 0.05$, Fig. 2E). Restraint had no effect on pelage score ($P > 0.05$, Fig. 2E).

Body Mass

Hamsters in short days adopted a short day phenotype evidenced by decreased body mass compared to long days ($U = 271$, $P = 0.001$, Fig. 3A). Dim light at night blocked the short-day mediated decreased in body mass; dim light at night increased body mass compared to dark nights ($U = 764$, $P = 0.002$, Fig. 3A and C). Body mass was reduced in restrained compared to unrestrained hamsters ($U = 235$, $P = 0.000$, Fig. 3A).

Testosterone Concentrations

Short-day hamsters displayed reduced testosterone concentrations compared to their long-day counterparts ($U = 264.5$,

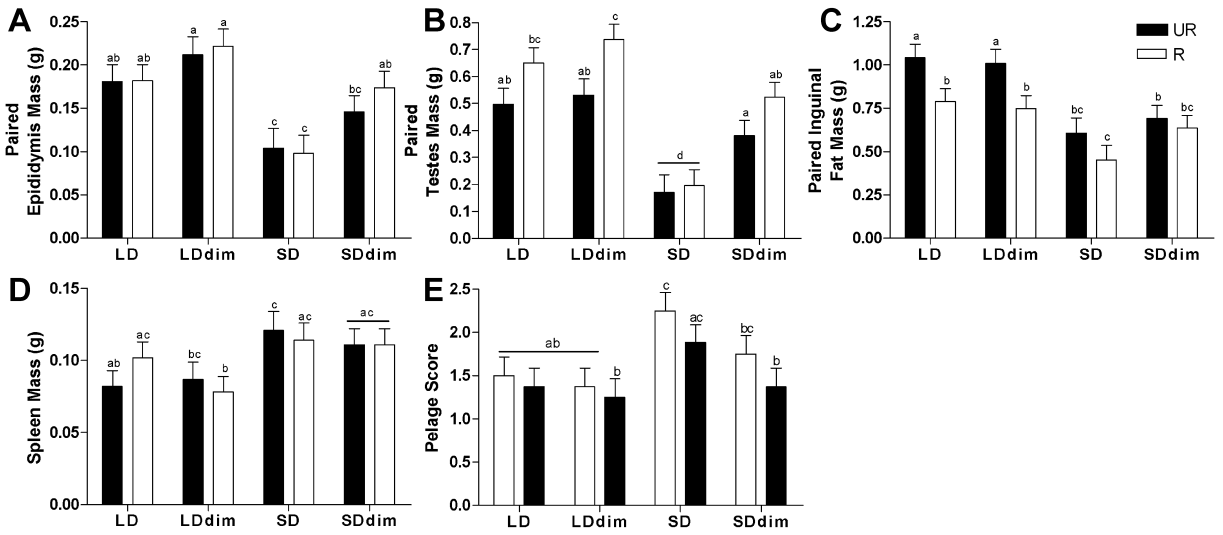

Figure 2. Short day Siberian hamsters (*Phodopus sungorus*) had regressed gonads only in dark nights. Mean (±standard error of the mean (SEM)) final paired epididymides mass (A), paired testes mass (B), paired inguinal fat pad mass (C), spleen mass (D), and pelage score (E), different letters indicate significance at $P < 0.05$. LD, long days and dark nights; SD, short days and dark nights; LDdim, long days and dim light at night; SDdim, short days and dim light at night; UR, unrestrained; R, restrained.

$P = 0.001$, Fig. 3B). Dim light at night increased testosterone concentrations in both short and long-day hamsters ($U = 751$, $P = 0.000$, Fig. 3B and D). Restraint did not affect testosterone concentrations ($P > 0.05$, Fig. 3B).

DISCUSSION

Artificial light at night is increasingly prevalent. As human populations expand outward from urban centers, they increasingly encroach on the habitats of small mammals and could provoke stress responses. Our results indicate that light at night impairs the development of a short day phenotype which encompasses a suite of behavioral and physiological adjustments.

As expected, dim light at night impaired cell-mediated immunity (Bedrosian et al., 2011). Although the mechanism for light at night effects on immune function remains undetermined, a likely candidate, although not assayed in the current study, is disrupted pineal melatonin. Light as low as ~1 lux during the subjective night is sufficient to suppress pineal melatonin in Syrian hamsters (Brainard et al., '82). Nighttime melatonin levels decrease with exposure to less than 1 lux of nighttime lighting, mimicking levels of light exposure recorded in free-living urban-dwelling songbirds (Dominoni et al., 2013b). DTH is a T-cell-mediated immune response; melatonin influences the immune system generally, and particularly T-cells (Huber et al., '76; Maestroni, '93). Impairment of the DTH response by dim light at night was most pronounced in short day hamsters that typically enhance responses relative to long day animals (Bilbo et al., 2002).

Restraint stress also impaired cell-mediated immunity, although a cell-mediated immune enhancement was retained in short day compared to long day hamsters. Chronic stress is often immunosuppressive (Dhabhar and McEwen, '97). In short day hamsters the combination of dim light at night and restraint decreased cell-mediated immunity at several time points compared to only restraint or only dim light at night among short day hamsters. These results suggest that dim light at night and stress have the potential to negatively affect species survival, especially during the short days of winter, an already challenging time for survival (Nelson and Demas, '97). Inflammatory responses to DNFB induce a reliable T-cell mediated response in Siberian hamsters (Bilbo et al., 2002; Prendergast et al., 2013). Given that a portion of the hamsters did not exhibit any inflammation, including them in analysis would provide inaccurate results.

Hamsters were moved from group housing to individual housing at the beginning of restraint. Although housing a male and a female together can interfere with the short day enhancement of DTH in Siberian hamsters, social housing of two males does not alter the photoperiodic or DTH responses compared to single-housed males (Weil et al., 2007). Additionally, housing of two males or a male and female pair elevates cortisol concentrations, suggesting that social housing may serve as a stressor to Siberian hamsters (Demas et al., 2004). Thus, moving hamsters to single housing after development of photoperiodic responses provided a baseline to assess the effects of photoperiod, dim light at night, and restraint on DTH responses.

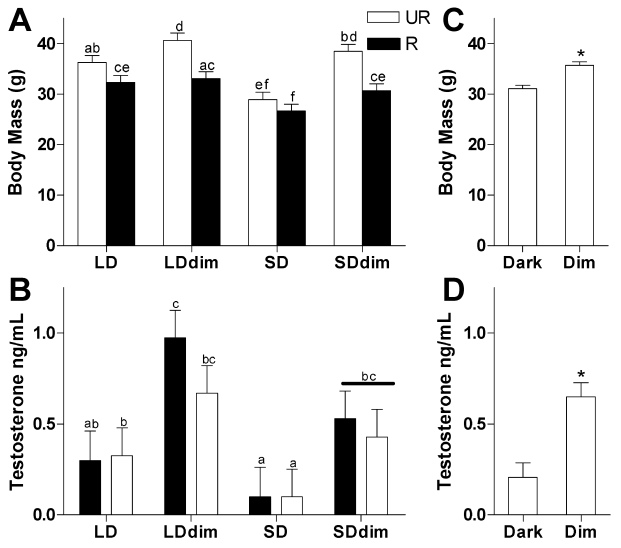

Figure 3. Overall, body mass was reduced by restraint and short days; testosterone concentrations were reduced by short days and increased by dim light at night. Mean (±standard error of the mean (SEM)) body mass at the end of restraint (A), different letters indicate significance at $P < 0.05$. Mean final testosterone concentrations (B), different letters indicate significance at $P < 0.05$. Mean body mass at the end of restraint in dark nights versus dim light at night (C), * indicates significance at $P < 0.05$. Mean final testosterone concentrations at the end of restraint in dark nights versus dim light at night (D), * indicates significance at $P < 0.05$. LD, long days and dark nights; SD, short days and dark nights; LDdim, long days and dim light at night; SDdim, short days and dim light at night; UR, unrestrained; R, restrained.

Dim light at night reduced the short day enhancement of cell-mediated immunity; dim light at night also blocked regression of reproductive organs. This was likely mediated, at least in part, by the maintenance of testosterone concentrations in hamsters exposed to dim light at night regardless of day length. Similarly, regrowth of reproductive organs can be advanced in European blackbirds by exposure to light at night (Helm et al., 2013). Melatonin induces gonadal regression and reduces testosterone concentrations in Syrian hamsters similar to the effects of short day exposure (Maywood et al., '90). Whereas testosterone is generally considered immunosuppressive (Hamilton and Zuk, '82; Folstad and Karter, '92), testosterone enhances some aspects of immune function in Siberian hamsters (Bilbo and Nelson, 2001). However, the presence of testosterone does not alter the short day enhancement of the DTH response in hamsters; therefore, compromised DTH response under light at night likely is independent of elevated testosterone (Prendergast et al., 2005). Dim light at night produces an intermediate short day phenotype, though the mechanism remains unspecified.

Although we did not assess changes in activity patterns or period length, dim light at night can alter these parameters. Songbirds dwelling in urban areas have an advanced activity onset compared to birds in rural areas, as well as weaker circadian oscillation of locomotor activity (Dominoni et al., 2013a, c). Similarly, light at night decreases the strength of the 24-hr rhythm in Siberian hamsters and C57bl/6 mice though they remain entrained (Bedrosian et al., 2013). The urban environment, particularly light at night, can modify biological rhythms in both wild and laboratory animals which may have negative implications for reproductive and survival success.

The failure to adopt a short day phenotype during the winter could pose a serious threat to individuals' fitness by causing a mismatch between reproductive and immune function trade-offs. Short days induce gonadal regression and improve immune function in Siberian hamsters (Bilbo et al., 2002). The shift in energy away from reproduction and towards immune function is likely beneficial to survival (Martin et al., 2008). The circadian system is important for appropriate adaptations of photoperiod mediated changes in behavior and physiology and disruption of the circadian system impairs animal fitness, that is, survival and successful reproduction (Emerson et al., 2008). Light at night, therefore has negative effects on small mammals, including impaired adoption of appropriate photoperiodic responses and subsequent annual shifts in immune priorities. These results suggest that with continued expansion of urban populations and nighttime lighting, numbers of photoperiodic mammals may continue to decline.

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