



Short Communication

Artificial light at night alters delayed-type hypersensitivity reaction in response to acute stress in Siberian hamsters



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ABSTRACT

Several physiological and behavioral processes rely on precisely timed light information derived from the natural solar cycle. Using this information, traits have adapted to allow individuals within specific niches to optimize survival and reproduction, but urbanization by humans has significantly altered natural habitats. Nighttime light exposure alters immune function in several species, which could lead to decreased fitness or survival, particularly in the face of an environmental challenge. We exposed male Siberian hamsters (*Phodopus sungorus*) to five lux of light at night for four weeks, and then administered six hours of acute restraint stress. Delayed-type hypersensitivity (DTH) response was assessed immediately following stress. Acute restraint increased the DTH reaction in dark nights, but exposure to nighttime light prevented this response. Exposure to light at night prolonged the DTH response in non-stressed control hamsters. These results suggest that light pollution may significantly alter physiological responses in Siberian hamsters, particularly in response to a salient environmental challenge such as stress.

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1. Introduction

Individuals display adaptations of circadian and seasonal rhythms in physiological and behavioral responses to maximize survival and reproduction. For example, organisms use light and dark information to determine time of day, which is essential to constrain activity to the period during which the threat of predation is lowest, and to determine time of year, which is critical to successful reproduction. Light information detected by the retina and relayed to the suprachiasmatic nucleus in the hypothalamus is the primary mechanism species use to align physiology and behavioral processes to the environment. Over the past ~120 years, however, urbanization by humans has dramatically altered natural habitats by creating pervasive light pollution. In the absence of artificial light, a full moon produces only about 0.1–0.3 lux, whereas an overcast night sky is about 0.0003–0.0001 lux (Rich and Longcore, 2006). Streetlights increase ambient illumination to approximately 5–60 lux, depending on the distance to the light source (Gaston et al., 2012). Urban areas are presumably even brighter still.

Exposure to artificial light at night (LAN) disrupts circadian and seasonal adaptations, which potentially decrease fitness. For exam-

ple, the timing of activity and reproductive behavior is altered in animals living near streetlights; songbirds alter mating calls and lay eggs earlier than conspecifics living deep in the forest (Kempenaers et al., 2010). Street lights disturb the commuting patterns of bats and they show no evidence of habituating over time to the artificial light (Stone et al., 2009). Beach mice living near populated coastlines have impaired foraging behavior (Bird et al., 2004). In the laboratory, immune function can be used to study the effects of nighttime lighting. LAN alters cell-mediated and humoral immune function in hamsters (Bedrosian et al., 2011), Japanese quail (Moore and Siopes, 2000), cockerels (Kirby and Froman, 1991), and rats (Oishi et al., 2006). It is possible that these effects may be more pronounced under harsh environmental conditions, the demands of limited resources, or stressful experience. Under these circumstances, decreased immune function could significantly affect survival.

Acute stressors enhance some aspects of immune function, specifically the antigen-specific, cell-mediated immune response called delayed-type hypersensitivity (DTH), whereas chronic stress suppresses these responses (Dhabhar and McEwen, 1997). In this experiment, we exposed Siberian hamsters to 5 lux dim LAN for four weeks and then administered six hours of acute restraint stress. DTH response, a measure of cell-mediated immune function, was assessed following the restraint stress to determine how the combined challenges of stress and exposure to nighttime light influence the DTH response. We also measured body mass, splenic mass, and testes mass to determine whether LAN influ-

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enced these photoperiod-responsive measures. We hypothesized that LAN would interact with acute restraint stress to alter the DTH reaction.

2. Methods

2.1. Animals

Adult (>8 weeks of age) male Siberian hamsters (*Phodopus sungorus*) were obtained from our breeding colony at The Ohio State University. Hamsters were individually housed in polypropylene cages (30 × 15 × 14 cm) at a constant temperature (22 ± 2 °C) and relative humidity (50 ± 5%). Food (Harlan Teklad 8640, Indianapolis, IN, USA) and filtered tap water were available *ad libitum*. All of the hamsters were born and matured to adulthood in a standard long-day 16 h light-8 h dark cycle (150 lux/0 lux). Half of the hamsters ($N = 16$) remained in the standard light cycle upon reaching adulthood and the other half ($N = 16$) were exposed to a 16 h light-8 h dim light cycle (150 lux/5 lux). Overhead daytime lights (150 lux) were illuminated from 23:00 to 15:00 h in both groups. Both the overhead bright lights and the dim night light were standard fluorescent bulbs emitting “cool white” light composed of wavelengths distributed across the visible spectrum. The small dim fluorescent night light was obtained from GE (Model 10059-F8T5/CW). Light intensity was measured at cage level. All experimental procedures were approved by The Ohio State University Institutional Animal Care and Use Committee and performed in accordance with NIH guidelines.

2.2. Restraint stress

Half of the hamsters were placed in a ventilated Plexiglas restraint tube for 6 h during the light phase (09:00–15:00 h) prior to DTH challenge the next morning. The other half of the hamsters remained in the home cage.

2.3. Delayed-type hypersensitivity (DTH)

After four weeks of exposure to nighttime light, DTH reaction was assessed as previously described (Bilbo and Nelson, 2003). DTH is an ecologically valid *in vivo* assay of cell-mediated immune function, including T-cell-mediated inflammation and antigen processing and presentation (Nelson et al., 2010). Briefly, DTH was induced by sensitization to, and later challenge with, the antigen 2,4-dinitro-1-fluorobenzene (DNFB; Sigma). Hamsters were sensitized by applying 25 µl of DNFB (0.5% in 4:1 acetone to olive oil vehicle) to the shaved skin of the dorsum on two consecutive days. Seven days later baseline pinnae thickness was measured with a constant loading dial micrometer (Mitutoyo, Tokyo), and then hamsters were challenged on the right pinna with 20 µl of 0.2% DNFB in vehicle, while the left pinna was treated with vehicle solution alone. The thicknesses of both pinnae were measured every day for the next 6 days. All measurements were made at the same time of day (between 08:30 and 10:00 h) and each hamster was brought into the procedure room individually to minimize potential stressors. Hamsters were lightly anesthetized with isoflurane vapors to perform sensitization, challenge, and measurements.

2.4. Body tissues

Twenty four hours after DTH measurements were completed, body mass was assessed and then hamsters were euthanized by rapid decapitation. Testes and spleens were dissected and weighed.

2.5. Statistical analyses

Repeated-measures ANOVA was used to analyze DTH response data between hamsters exposed to dark or dim light at night and restraint or no restraint. Two-way ANOVA was used to analyze body mass and tissue weights, with light condition and restraint condition as the independent variables. Significant main effects were followed up with Fisher's post-hoc comparisons. Statistics were performed using Statview 5.0.1 for Windows. Mean differences were considered statistically significant when $p < 0.05$.

3. Results

Overall, there were significant main effects of restraint condition ($F_{1,30} = 15.37$, $p = 0.0001$), and day post-DTH challenge ($F_{6,196} = 5.85$, $p < 0.0001$) on pinna thickness, as well as a significant interaction of lighting condition and restraint ($F_{1,30} = 4.40$, $p = 0.04$; Fig. 1A).

The interaction of lighting by restraint is depicted in Fig. 1B. Hamsters exposed to dark nights and restraint stress had a greater DTH reaction than control hamsters in either dark or dim lighting conditions (post-hoc, $p < 0.0001$ and $p < 0.05$, respectively). Hamsters exposed to dim LAN and restraint stress had a greater DTH response than control hamsters exposed to dark nights (post-hoc, $p < 0.01$).

We compared the effect of exposure to nighttime light on the DTH reaction. Exposure to dim light at night provoked a prolonged DTH response compared to exposure to dark nights in non-restrained hamsters. There was a main effect of lighting condition ($F_{1,30} = 5.67$, $p = 0.02$) and a main effect of day post-challenge ($F_{6,196} = 2.95$, $p = 0.01$; Fig. 1C). Within restrained hamsters, exposure to light at night had no effect. But as expected, there was an effect of time post-challenge ($F_{6,196} = 3.28$, $p = 0.006$; Fig. 1D).

We also compared the effect of acute restraint on the DTH reaction within lighting conditions. As expected, acute restraint significantly increased DTH response in hamsters exposed to dark nights ($F_{1,30} = 22.76$, $p < 0.0001$). The increase in pinna thickness was significantly greater in restrained hamsters compared to non-restrained hamsters at days 3, 4, 5, and 6 (post-hoc, all $p < 0.05$). There was also a main effect of day post-challenge ($F_{6,196} = 3.45$, $p = 0.004$; Fig. 1E). In contrast, acute restraint did not affect the DTH reaction in hamsters exposed to dim light at night. There was no significant effect of restraint and no interaction effect of restraint and day post-challenge, but there was a main effect of day ($F_{6,196} = 2.60$, $p = 0.02$; Fig. 1F). There were no significant effects of restraint or light condition on body mass, testes, or spleen mass.

4. Discussion

Our results indicate that both exposure to dim nighttime lighting and acute restraint stress can influence the DTH response in Siberian hamsters. This suggests that chronic exposure to light pollution, even light intensities as low as 5 lux, may be sufficient to significantly alter physiological processes, which could potentially influence fitness and survival by decreasing host defense or shunting energy towards other processes. It will be important to further investigate the role of LAN on physiological processes in a variety of different species, as organisms vary in their sensitivity to light. Siberian hamsters are photoperiodic rodents and very sensitive to low level light. The observation that body mass, testes, and spleen mass—all photoperiod-responsive measures—were not influenced by the manipulations in this experiment suggests that there was no overt photoperiod adjustments.

We observed subtle effects of LAN on DTH reaction. The mechanism whereby LAN influences DTH response remains undeter-

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