General and Comparative Endocrinology 197 (2014) 56–64

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Dim light at night disrupts the short-day response in Siberian hamsters

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ARTICLE INFO

Article history:
Received 6 August 2013
Revised 3 December 2013
Accepted 9 December 2013
Available online 19 December 2013

Keywords:
Seasonality
Photoperiodism
Light pollution
Pelage
Immune function

ABSTRACT

Photoperiodic regulation of physiology, morphology, and behavior is crucial for many animals to survive seasonally variable conditions unfavorable for reproduction and survival. The photoperiodic response in mammals is mediated by nocturnal secretion of melatonin under the control of a circadian clock. However, artificial light at night caused by recent urbanization may disrupt the circadian clock, as well as the photoperiodic response by blunting melatonin secretion. Here we examined the effect of dim light at night (dLAN) (5 lux of light during the dark phase) on locomotor activity rhythms and short-day regulation of reproduction, body mass, pelage properties, and immune responses of male Siberian hamsters. Short-day animals reduced gonadal and body mass, decreased spermatid nuclei and sperm numbers, molted to a whiter pelage, and increased pelage density compared to long-day animals. However, animals that experienced short days with dLAN did not show these short-day responses. Moreover, short-day specific immune responses were altered in dLAN conditions. The nocturnal activity pattern was blunted in dLAN hamsters, consistent with the observation that dLAN changed expression of the circadian clock gene, *Period1*. In addition, we demonstrated that expression levels of genes implicated in the photoperiodic response, *Mel-1a* melatonin receptor, *Eyes absent 3*, *thyroid stimulating hormone receptor*, *gonadotropin-releasing hormone*, and *gonadotropin-inhibitory hormone*, were higher in dLAN animals than those in short-day animals. These results suggest that dLAN disturbs the circadian clock function and affects the molecular mechanisms of the photoperiodic response.

1. Introduction

Development of seasonal adaptations is a widespread strategy to increase survival and reproductive success for living organisms. During winter, many small mammals, such as Siberian hamsters, limit reproductive activity by regressing their gonads, decreasing body mass, molting to a winter pelage with white fur color and high fur density, and adjusting immune functions (Walton et al., 2011; Scherbarth and Steinlechner, 2010; Goldman, 2001). The annual cycle of changing day length (photoperiod) is commonly used as a signal of the approaching season. Photoperiodic information is encoded by the central circadian clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus (Hastings and Herzog, 2004). SCN regulates the synthesis and release of melatonin from the pineal gland at night; therefore, differences in the duration of melatonin secretion represent differences in day length (Pevet and Challet, 2011). Melatonin targets several brain regions to affect the phase of peripheral circadian clocks, as well as the central clock in the SCN, by altering expression of circadian clock genes (Pevet and Challet, 2011). Among the target sites of melatonin, the pars tuberalis (PT) of the pituitary stalk plays a key role in the photoperiodic pathway (Dardente, 2012).

In mammals, a long-day signal rapidly induces the strong peak of the transcription factor *Eyes absent 3* (*Eya3*) in the PT (Dardente et al., 2010; Masumoto et al., 2010). EYA3 contributes to thyroid-stimulating hormone (TSH) synthesis in the PT by activating transcription of *TSH β subunit* (*TSHβ*). TSH acts on TSH receptor (TSHR)-expressing cells in the basal hypothalamus to increase thyroid hormone (T_3) availability, leading to seasonal gonadal growth and body mass increase (Barrett et al., 2007). Secretion of gonadotropin from the pituitary gland is stimulated by a hypothalamic neuropeptide gonadotropin-releasing hormone (GnRH) (Stevenson et al., 2012). In contrast, gonadotropin-inhibiting hormone (GnIH) in birds suppresses synthesis and release of gonadotropins by acting on GnRH neurons as well as directly on the pituitary gland

Abbreviations: dLAN, dim light at night; DNFB, 2,4-dinitro-1-flourobenzene; DTH, delayed-type hypersensitivity; *Eya3*, *Eyes absent 3*; GnIH, gonadotropin-inhibiting hormone; GnRH, gonadotropin-releasing hormone; LD, long days; PT, pars tuberalis; LPS, lipopolysaccharide; *Per1*, *Period1*; RFRP, RFamide-related peptide; SCN, suprachiasmatic nuclei; SD, short days; TSH, thyroid-stimulating hormone; TSHR, TSH receptor.

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http://dx.doi.org/10.1016/j.ygcen.2013.12.005

(Ubuka et al., 2008; Ciccone et al., 2004). As in birds, mammalian GnIH orthologs (RFamide-related peptide gene: RFRP) are expressed in the hypothalamus and the inhibitory effects of these orthologs on gonadotropin secretion has been demonstrated in Siberian and Syrian hamsters housed in long days (Kriegsfeld et al., 2006; Ubuka et al., 2012); however, stimulatory effects of GnIH on the gonadotropic axis has also been reported in short-day hamsters (Ancel et al., 2012; Ubuka et al., 2012), consistent with the observation that expression of *GnIH* is increased in long days in Siberian hamsters (Ubuka et al., 2012). These disparate results suggest photoperiodic history-dependent responces to GnIH (Henson et al., 2013).

Although environmental factors such as temperature can vary from year to year, annual changes in day length follow a predictable pattern. However, recent urbanization activity by humans has increased the prevalence of artificial light at night, rapidly changing the natural environment to which organisms must adjust (Hölker et al., 2010). Exposure to light at night alters the circadian system and affects many physiological responses (Navara and Nelson, 2007). Low-level lighting (1.08 lx) is sufficient to suppress nocturnal secretion of melatonin in Syrian hamsters (Brainard et al., 1982), and exposure to night-time dim light (5 lux), which is comparable to the levels of night surrounding urban centers, alters the expression of circadian clock proteins (Bedrosian et al., 2013) and adaptive behaviors in rodents (Fonken et al., 2012; Fonken and Nelson, 2013; Bedrosian et al., 2011a, 2011b). Even nighttime light below the intensity of moonlight can affect the circadian organization and photoperiodic response (Evans et al., 2009, 2012; Gorman and Elliott, 2004; Gorman et al., 2006). Indeed, effects of artificial night lighting on daily activity rhythms and seasonal timing of reproduction have been suggested in wild animals (Dominoni et al., 2013; Kempenaers et al., 2010; Stone et al., 2009; Bird et al., 2004).

In the present study, we examined the effects of dim light at night (dLAN) on the locomotor activity rhythm and the short-day regulation of reproduction, body mass, pelage properties, and immune responses of Siberian hamsters. We also examined whether dLAN altered expression of the circadian clock gene *Period1* (*Per1*) and genes required for the photoperiodic response, melatonin receptor (*Mel-1a*), *Eya3*, *Tshr*, *GnRH*, and *GnIH* in the hypothalamus and PT. We hypothesized that dLAN disrupts seasonal adaptations through alteration of the molecular pathway of the photoperiodic system.

2. Materials and methods

2.1. Animals

Siberian hamsters (*Phodopus sungorus*) used in this study were bred in our colony at The Ohio State University. Male hamsters were weaned during the light phase at 21–24 d of age and reared to adulthood in long-day conditions (LD: 16 h light (150 lux)-8 h dark (0 lux), with lights illuminated from 23:00 to 15:00 h EST) at a constant temperature of 21 ± 2 °C and relative humidity of 50 ± 10%. At 93–114 d of age, hamsters were placed into short-day conditions (SD: 8 h light (150 lux)-16 h dark (0 lux), with lights illuminated from 7:00 to 15:00 h EST, $n = 18$), dim light at night in short-day conditions (SD-dLAN: 8 h light (150 lux)-16 h dim light (5 lux), with bright lights illuminated from 7:00 to 15:00 h EST, $n = 17$) or maintained in long-day conditions ($n = 17$). Hamsters were individually housed in polypropylene cages (30 × 15 × 14 cm) and had *ad libitum* access to food (Harlan Teklad Rodent Diet 8640; Indianapolis, IN, USA) and filtered tap water. All procedures were approved by the Ohio State University Institutional Animal Care and Use Committee and comply with guidelines established by the National Instituted of Health published by the Institute of Laboratory Animal Resources (U.S.) (2011).

2.2. Tissue collection and reproductive and somatic measures

After four or eight weeks of photoperiodic exposure, or after the immune response experiments (~20 weeks of photoperiodic exposure), hamsters were anesthetized with isoflurane vapors ($n = 7$–10, for each condition), and body mass was assessed. At the same time, the fur color of the animals was scored according to the stage classification (Figala et al., 1973). Fur color 1 represents the summer coat (dark brownish fur). Fur color 6 represents the winter coat (white fur). Pelage density was also determined by shaving and weighing a 1 cm^2 patch of fur obtained from the anterior dorsal surface of animals. Then, hamsters were rapidly decapitated; testes, epididymides, gonadal fat pads, and seminal vesicles were removed and weighed. Brains from animals exposed to each photoperiod for eight weeks were removed, placed in RNAlater (Ambion TX, USA), and stored at 4 °C to maintain mRNA integrity for gene expression analysis. Body mass and pelage scores were also assessed in all experimental animals after 4, 8, 10, and 14 weeks of photoperiodic exposure until animals were killed in each experiment. All procedures were conducted between 14:00 and 15:00 h EST. In SD-dLAN groups, animals with paired testes masses within 2 standard deviations of the mean of LD animals of corresponding exposure duration were judged as reproductive. There were no animals that were judged as reproductive in the SD group.

2.3. Spermatid nuclei and sperm counts

Paired testes and epididymides were minced with scissors and homogenized by using a blender for 30 and 45 s, respectively, in 25 and 50 mL, respectively, of a buffer composed of 0.15 M sodium chloride containing 0.05% Triton-X 100 (Sigma) with 0.025 mM thimerosal (Sigma). Testicular spermatid nuclei and epididymal sperm in the homogenate were counted on a hemocytometer. Duplicate determinations were made for each homogenate.

2.4. Delayed-type hypersensitivity (DTH)

After 8 weeks of photoperiodic exposure, DTH was induced by application of the antigen, 2,4-dinitro-1-flourobenzene (DNFB; Sigma). On day 1 and day 2, hamsters ($n = 8$–9, for each conditions) were sensitized by applying 25 μL of DNFB (0.5% wt/volume in 4:1, acetone/olive oil vehicle) to the shaved skin on the dorsum. Seven days later hamsters were anesthetized and baseline pinnae thickness was measured by using a constant loading micrometer (Mitutoyo, Tokyo, Japan). Twenty microliters of DNFB (0.2% wt/volume in 4:1, acetone/olive oil vehicle) was applied to the skin of the dorsal surface of the right pinna, and left pinna was treated with 20 μL of vehicle. Pinna thickness was measured every 24 h for the next 5 d at 11:00 h EST, and all measurements were made on the same relative region of the pinna.

2.5. Lipopolysaccharide (LPS)-induced responses

Approximately 10 weeks after DTH measurements, hamsters ($n = 7$–8, for each conditions) were implanted intraperitoneally with radiotelemetric transmitters (Mini-Mitter, OR, USA) under anesthesia and allowed to recover for one week. Cages were placed on TR-3000 receiver boards and connected to DP-24 DataPorts (Mini-Mitter) to continuously collect activity and temperature data in 30 min intervals. Right after data collection at 14:00 h EST (1 h before light–dark or light-dim light transition), hamsters were given intraperitoneal injections of 0.1 mL saline to establish the baseline activity and temperature information. Twenty-four hours