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Role of light wavelengths in synchronization of circadian physiology in songbirds

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HIGHLIGHTS

- Compared responses to light wavelengths between nonmigratory and migratory songbirds
- Birds use wavelengths in photoperiodic timing regardless of their breeding latitude.
- Short light wavelength is interpreted as day and long light wavelength as the night.
- Activity levels and melatonin secretion are phase related, except in migratory state.
- Daily cortisol levels were not significantly affected by the spectral regimes.

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ABSTRACT

This study investigated whether at identical duration and equal energy level birds presented with short (450 nm; blue, B) and long (640 nm; red, R) light wavelengths would differentially interpret them and exhibit wavelength-dependent circadian behavioral and physiological responses, despite the difference in their breeding latitudes. Temperate migratory blackheaded buntings (*Emberiza melanocephala*) and subtropical non-migratory Indian weaverbirds (*Ploceus philippinus*) initially entrained to 12 h light:12 h darkness (12L:12D; L = 0.33 μM/m^2/s, D = 0 μM/m^2/s) in two groups of each, groups 1 and 2, were subjected to constant light (LL, 0.33 μM/m^2/s), which rendered them arrhythmic in the activity behavior. They were then exposed for about two weeks each to 12B:12R and 12R:12B (group 1) or 12R:12B and 12B:12R (group 2) at 0.33 μM/m^2/s light energy level. Blue and red light periods were interpreted as the day and night, respectively, with activity and no-activity in non-migratory weaverbirds or activity and intense activity (*Zugunruhe*, migratory night restlessness) in the migratory buntings. Consistent with this, plasma melatonin levels under B:R, not R:B, light cycle were low and high in blue and red light periods, respectively. A similar diurnal pattern was absent in the cortisol levels, however. These results show an important role of light wavelengths in synchronization of the circadian clock governed behavior and physiology to the photoperiodic environment, and suggest that photoperiodic timing might be a conserved physiological adaptation in many more birds, regardless of the difference in breeding latitudes, than has been generally envisaged.

1. Introduction

Most circadian and photoperiodic studies have shown the importance of daylight in controlling daily and seasonal functions in vertebrates [14]. In the natural 24-h light environment, however, the day and night components are linked by corresponding change in the duration, and by relative differences in the intensity and spectrum of light available. Also, there are changes in the illumination levels at night, but seem perhaps buffered against many fold brighter illumination during the day. In general, circadian and photoperiodic responses in birds depend on the subjective interpretation of illumination during the day and night, hence day–night illumination contrast, rather than on the day light intensity alone (Japanese quail, *Coturnix c. japonica* [18,19]; blackheaded bunting, *Emberiza melanocephala* [12]; Indian weaver bird, *Ploceus philippinus* [30]. However, moonlight or an artificial light at equivalent intensity has been shown to influence the circadian responses including melatonin secretion in fishes [7,23].

The spectral composition of daylight can affect the circadian and photoperiodic responses in vertebrates, including birds [25]. In laboratory, 12 h short:12 h long light wavelengths (3400: 2900 K color temperatures) applied at equal energy level (44 mW cm^2) is shown to synchronize circadian activity rhythms in zebra finches, *Taeniopygia guttata* [6]. A similar synchronizing effect of light wavelength on

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circadian activity rhythms has also been shown in brambling, *Fringilla montifringilla* [22], common redpoll, *Carduelis f. flammea* [21] and blackheaded bunting, *E. melanocephala* [15]. Migratory bramblings and redpolls exposed to a 12 h:12 h light paradigm and presented with short (blue, B) and long (red, R) light wavelengths at equal energy levels, were synchronized to B:R light cycles [21]. Synchronizing effect of daily oscillations in spectral distribution of sunlight on avian circadian rhythms has also been shown in the field studies [11]. Further, circadian control of wavelength-induced migratory behavior (*Zugunruhe*, migratory restlessness), evidenced by an intense nighttime activity and wing-whirring in caged birds [8], is shown to be modulated by the light spectrum through changes in melatonin secretion [22]. Shorter light wavelengths (430–560 nm; blue-green light) suppress night melatonin levels with delayed melatonin peak in several species, including fish, chicken, rat and human [4,5,35]. The photoperiodic clock responds to light wavelengths in circadian phase-dependent manner in the migratory blackheaded and redheaded buntings [13,15,24].

The effects of light wavelengths on avian circadian and photoperiodic responses, independent of the duration and intensity of the light period, have been scarcely investigated. Further, light responses may differ between high and low latitude breeding species, for they would have conserved annual itineraries evolved from a close interaction between the endogenous timing program and surrounding light environment [16]. For example, testis maturation–regression cycle under stimulatory long days occurred at a faster rate in temperate migratory redheaded buntings, compared to that in the subtropical Indian weaverbirds [16].

Here, we tested whether songbirds belonging to different family groups that are phylogenetically close [2] but adapted to the different latitudes and exhibit differences in the life history states, would respond similarly and exhibit wavelength-dependent circadian responses if they were presented with short and long light wavelengths at identical duration and equal irradiance (energy level). Specifically, we compared synchronization of circadian behavioral and physiological responses between temperate migratory blackheaded buntings (family Emberizidae) and subtropical Indian weaverbirds (family Ploceidae), that were circadianly arrhythmic and presented with B:R and R:B spectral regimes at equal energy levels in a 12 h:12 h paradigm. These species have distinct differences in their annual life history states (migratory vs. resident), although they are phylogenetically closer, as revealed by the homogeneity of karyotypes of the two bird groups [2]. The prediction was that both species would respond similarly, and interpret 12 h blue and red light periods as the day and night, respectively, should the spectral sensitivity of photoreceptors mediating photoperiodism was a conserved trait among photoperiodic birds.

2. Methods

2.1. Animals and maintenance

This study included two photoperiodic songbirds, the blackheaded bunting (*E. melanocephala*) and Indian weaver bird (*P. philippinus*), which breed at distinctly different latitudes but share the same latitudes and environment for almost half of the year [1]. Bunting is a Palearctic-Indian migrant, with the breeding grounds in west Asia and east Europe. It arrives in India (~25°N) in late September/October, overwinters, and returns to its breeding grounds located around ~40°N in late March/April [1]. On the other hand, Indian weaver birds are a resident species.

For this study, birds were captured in late February, brought to the laboratory and acclimated to captive conditions for a week in an outdoor aviary (size = 3 × 2.5 × 2.5 m). Thereafter, they were moved indoors and kept under short photoperiods (8L:16D), in which they remain unstimulated and maintain photosensitivity, until the beginning of the experiment. The food (seeds of *Setaria italica* and *Oryza sativa*) and water were provided ad libitum.

2.2. Experiment

Photosensitive birds (buntings: n = 14; weaverbirds: n = 18) were housed individually in activity cages (60 × 35 × 45 cm) and placed inside the photoperiodic boxes (size, 75 × 50 × 70 cm) providing programmed light cycles by compact fluorescent bulbs (CFL; 14 W, 230 V, Phillips, India); temperature was maintained at 24 ± 2 °C during the experiment. Each box contained two bulbs, of which only one was lit at a time providing illumination at the level of cage floor. Desired light wavelength and intensity were obtained by covering the fluorescent light bulbs (14 Watt Philips) with neutral density and colored cinemoid filters (Rosco filters, Blanchard Works, Kangley Bridge Road, Sydenham, London, UK), respectively. These filters with transmission peaks at 450 nm (blue) and 640 nm (red) have been used in our previous studies [15]. The illumination was measured as energy levels, in PAR (μM/m^2/s) by a Q203 quantum radiometer (Macam Photomatrix Ltd., Scotland, UK).

Birds were initially kept on 8L:16D. On day 4, they were exposed to 12L:12D (L = 0.33 μM/m^2/s, D = 0 μM/m^2/s) by delaying the light offset by 4 h. After about two weeks, birds were released in constant light (LL) at identical light energy levels for two weeks to induce arrhythmicity in the circadian activity behavior. At this stage, they were distributed in two groups of each species (groups 1 and 2; n = 7 or 9 each) and subjected to spectral regimes (450 nm: blue, B; 640 nm: red, R) with identical light duration (12 h) and energy level (0.33 μM/m^2/s). The 12L:12D was replaced with 12B:12R (group 1) or 12R:12B (group 2). After two weeks, the wavelength pattern was phase-inversed for another two weeks; group 1 — 12R:12B, and group 2 — 12B:12R. This was done to remove bias, if any, in the interpretation of light wavelength by birds due to order of their presentation during an experiment. The duration of exposure to a particular light regime between buntings and weaverbirds differed by 2–3 days, to stagger the observation dates in two species for the sake of convenience.

2.3. Measurement of activity behavior

A Passive Infrared Motion Sensor with 12 m (40°) range (C & K Systems [Intellisense XJ-413T] Conrad Electronic, Germany, Haustier PIR-Melder), mounted on each cage detected the bird's movement, and transferred to a separate computer channel and stored in 5 min bins. The Chronobiology Kit software program (Stanford Software Systems, Stanford, CA) collected and analyzed the data on general activity of birds. The activity records (actograms) were presented in a double plot, with each day duplicated along the horizontal axis and subsequent days shown underneath in an increasing order. We calculated daily activity profile in hours and total activity in 12 h halves for each light conditions, as described in previous publications from our laboratory [15,30]. Briefly, activity counts per hour were calculated for a selected duration in a lighting exposure. Then, they were averaged for a number of days, and from this, daily activity profile (mean ± SE) was plotted. Similarly, the total activity was calculated for 12 h halves for same number of days in each light condition. Also, the circadian periodicity was tested for each bird by Chi square Periodogram analysis.

2.4. Measurement of blood melatonin and cortisol levels

2.4.1. Blood sample and plasma collection

Blood samples were taken at hours 6 and 18 relative to lights on under 12L:12D; these were designated as ZT6 and ZT18 samples (ZT0, zeitgeber time = lights on). The same sampling times were used in LL, in the absence of another phase marker of the daily oscillation. Samples of 100–125 μl of blood were collected in heparinized capillary tube by veni-puncture of the wing vein, and immediately centrifuged. The plasma was harvested and stored at −20 °C until assayed for melatonin and cortisol by ELISA. All the spectrophotometric readings were taken at the