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Original article

The influence of light wavelength on reproductive photorefractoriness in migratory blackheaded bunting (*Emberiza melanocephala*)

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Abstract — There are two effects of long day length on reproductive responses in birds, one is the photoinduction of gonadal growth and maturation and the other is the induction of gonadal regression and photorefractoriness. Although it is likely that the same photoreceptors are involved in the photoinduction of gonadal growth and the onset and maintenance of photorefractoriness, and so the influence of wavelength should be similar, this has not been investigated. Therefore, we investigated the influence of light wavelength on reproductive photorefractoriness in the migratory male blackheaded bunting held under long photoperiods. In mid May, when photoperiod was approximately 14L:10D (14 hours light:10 hours darkness), eight groups of sexually mature birds were moved indoors on an artificial photoperiod of 14L:10D (L - 450 lux, D - 0 lux). Then after 3 weeks, for six groups, a 4-h light period in the morning (zt 0–4; zt 0 [zeitgeber time 0] refers to the beginning of lights-on period) or in the evening (zt 10–14) was substituted with green (428 nm), red (654 nm) or white light at 16 ± 2 lux intensity. Of the remaining two groups, one was maintained on 14L:10D and the other transferred to 10L:14D; these served as controls. At the end of 4 weeks, all birds were found to have undergone testicular regression, irrespective of LD cycle they were exposed to. When these gonadally regressed birds were subjected to 16L:8D for another 4 weeks, to test their responsiveness to the stimulatory effects of long day lengths, only those exposed to 10L:14D and 14L:10D with a 4-h green light period showed testicular regrowth. On the other hand, birds exposed to 14L:10D with a 4-h white or red light period remained fully regressed, similar to 14L:10D controls. Except for some individual difference, there was no difference in response between the groups that received a 4-h light period in the morning and that received it in the evening. These results suggest that the wavelengths of light influence induction of buntings from the photosensitive state into the photorefractory state. Whereas the short light wavelengths facilitated recovery from the photorefractoriness, the long light wavelengths were more effective in maintaining the photorefractoriness.

bunting / photoperiod / photosensitivity / testis / wavelength

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1. INTRODUCTION

Day length regulates seasonal responses, namely reproduction and associated events, in a number of birds [7, 12]. In a long-day bird species, there are two effects of long day length on reproductive responses, one is the photoinduction of gonadal growth and maturation and the other is the induction of gonadal regression and photorefractoriness. During photorefractoriness, the photoperiodic response system does not translate light input into a neuroendocrine response as a result of positive switching off its ability to respond to the stimulatory effects of the long day length (for review see [6, 12, 21]). The photorefractory individuals respond to increasing spring and summer day lengths of the subsequent year after they have experienced decreasing (short) day lengths of autumn and winter during the intervening period of the year. A similar gonadal growth–involution–refractoriness cycle is induced under an artificial long day length (e.g. 14 h light:10 h dark, 14L:10D or 16L:8D), and the photorefractory individuals respond again to a long day length only after they were exposed for a period of about eight weeks to short day lengths, e.g. 8L:16D [12, 21]. A similar recovery of the photoperiodic sensitivity can occur even under a stimulatory day length if the intensity of the light period is reduced below the threshold of perception by the photoreceptors [29]. Bentley et al. [4] have reported that a decrease in light intensity alters the perception of the day length; starlings held on a 18L:6D at 3-, 13-, 45-lux intensities respond as if they were exposed to 11L:13D, 13L:11D, 16L:8D, respectively.

In birds, unlike mammals, the photoreceptors thought to mediate long day responses are in the hypothalamus and may be directly linked to the GnRH (gonadotrophin releasing hormone) neurones [27]. This might mean that the photosensitivity of the hypothalamic photoreceptors, which are necessary and sufficient for the detection of changes in day length, plays an important role in the photoperiodic regulation of reproductive cycle in birds. For over six decades, the role of light wavelengths is recognized in photoperiod-induced gonadal growth and development (for review see [15]). Long light wavelengths (e.g. red light) were found stimulatory and short light wavelengths (e.g. green or blue light) were found inhibitory or with no effects [3, 5, 9, 14, 18, 22, 25, 26]. Although it is likely that the same photoreceptors are involved in the photoinduction of the gonadal maturation and the onset of photorefractoriness, and so the influence of wavelength should be similar, this has not been investigated. Nonetheless, one might assume that in the long day paradigm a spectral cycle perceived by the photoreceptors as light period will maintain the photorefractoriness, and the one perceived by the photoreceptors as darkness will terminate the photorefractoriness or recover the bird's photoperiodic responsiveness to stimulatory effects of long day lengths. We have tested this assumption on highly photoperiodic blackheaded buntings (*Emberiza melanocephala*), by subjecting photostimulated individuals to a long photoperiod of which some light hours in the morning or in the evening were substituted with short or long light wavelength. Specifically, we report the effects on maintenance of the photoperiodic responsiveness to stimulatory effects of a long day length in reproductively mature blackheaded buntings (birds with large testes) exposed to 14L:10D (L - 450 lux, D - 0 lux) of which 4-h light period in the morning or in the evening was substituted with white, green (428 nm) or red (654 nm) light at 16 ± 2 lux intensity.

2. MATERIALS AND METHODS

The blackheaded bunting (*Emberiza melanocephala*) is a palaearctic-Indian-migratory finch that arrives in India (around 25° N) in October/November and returns to its breeding grounds (around 40° N) in late March/April [1]. The present study was done

on adult male birds that were caught from the overwintering flocks at 25° N and brought to our outdoor aviary (size = 3.8 × 1.7 × 2.3 m) at Lucknow University (27° N) in February. In captivity under natural day length (NDL) at 27° N, testes begin to recrudesce in April, attain maximum growth by the end of May/early June, begin to regress by the end of June/early July, and fully regress by August [20]. In laboratory, long day lengths (≥ 12 h) induce gonadotrophin secretion and testicular growth, and, following gonadal growth–involution cycle, photorefractoriness develops in birds that are photostimulated over a long period of time [13, 17, 30].

The present study began in mid May (day length = 14.3 h including 24–28 min of morning and evening civil twilight periods) from a batch of reproductively mature black-headed buntings (birds with large testes; testis volume, $TV = 66.98 \pm 1.86$, $N = 44$). If kept on NDL, testicular regression (one of the markers of the photorefractoriness) in these birds will occur by July/August, i.e. in the next 8–10 weeks. Eight groups of birds ($N = 5$–6 each) were initially exposed to 14L:10D (L = 450 lux, D = 0 lux) so that they synchronize to an artificial (square-wave form) lighting conditions. (We chose 14L:10D since it was close to the length of the day that buntings had experienced at this time in NDL.) After 3 weeks, for six groups, a 4-h period in the morning (zt 0–4; zt 0 [zeitgeber time 0] refers to the beginning of lights-on period) or in the evening (zt 10–14) of 14L was substituted with white, green (428 nm) or red (654 nm) light at 16 ± 2 lux intensity. We designed this kind of experiment (4 hours of wavelength treatment in the morning and evening of a long day length) in order to assess the phase-dependent effects of the light wavelength on photoperiodism in buntings. Furthermore, in another experiment, we (Malik, Rani and Kumar – unpublished) found that in photosensitive buntings held on 13L:11D at 8- and 37-lux intensities of green, white and red lights for 4 weeks, testes were not stimulated at 8 lux in any light, but at ~37 lux red light was stimulatory ($TV = 26.86 \pm 4.67$, $N = 9$). Therefore, for the present experiments, a light intensity at ~16 lux was chosen which was twice of the non-stimulatory intensity, noted above, but at the same time at this intensity we could possibly exclude an effect of light intensity per se on the maintenance of the photorefractoriness.

Of the remaining two groups on 14L:10D, one group was maintained on 14L and the other was transferred to 10L:14D; these served as controls. At the end of 4 weeks, all eight groups were subjected to 16L:8D for another 4 weeks to test their photoperiodic sensitivity as the consequence of exposure to different lighting conditions in an identical long day length.

Food and water were available ad libitum. The general experimental conditions (housing, lighting etc.) were the same as described in earlier publications [11, 17]. White and coloured light at 16 lux (with variation in the range of ± 2 lux over 24-h period) intensity were obtained by covering the fluorescent tube illuminating photoperiodic chamber with neutral density and coloured cinemoid filters (Rosco Filters: Blanchard Works, Kangley Bridge Road, Sydenham, London, England), respectively. The transmission peaks for green and red filters were at 428 and 654.4 nm, respectively.

Testis size was measured at the beginning (post 3-week synchronization to 14L:10D), after 4 weeks of exposure to different lighting conditions in 14L:10D and 10L:14D, and at the end of 4 weeks of exposure to 16L:8D. Testicular response, assessed by laparotomy under local anaesthesia [24], to different photoperiods was considered as the marker of the physiological sensitivity to day length. The dimensions of the left testis were recorded and testis volume was calculated from $4/3\pi ab^2$, where a and b denote half of the long and the short axes, respectively. The data, presented as means ± SEs

in Figures 1 and 2, were analysed by one-way analysis of variance with repeated measures (1-way RM ANOVA), followed by Student-Newman-Keuls post hoc test if ANOVA indicated the significance of difference. The means of different groups were also compared by paired and unpaired Student's t-test, as appropriate. Significance was taken at $p < 0.05$.

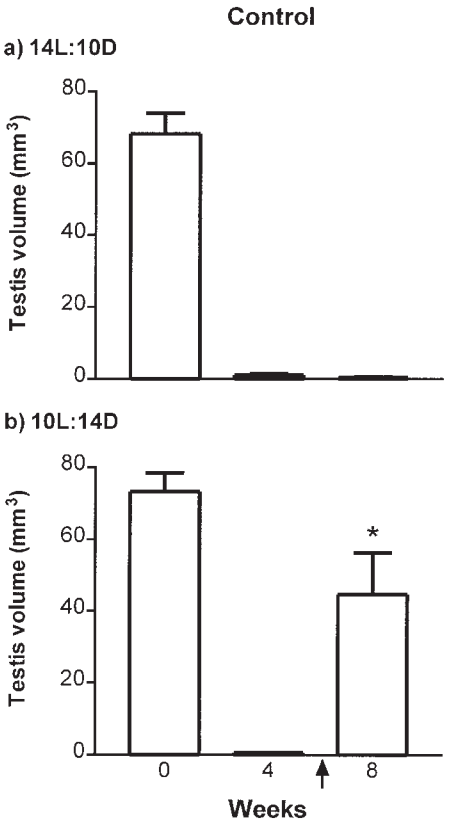

Figure 1. Data from control groups. Gonadally stimulated birds were first exposed to 14 hour light:10 hour darkness (14L:10D; L - 450 lux, D - 0 lux) for 3 weeks, and then they were either maintained on the same photoperiod (**a**) or transferred to 10L:14D (**b**) for another 4 weeks. Thereafter, both groups were subjected to 16L:8D for the next 4 weeks to test their sensitivity to stimulatory effects of long day length. Note restimulation of testes only in the group that was previously exposed to 10L:14D. Each bar represents the mean (± SE) testis volume for 5–6 birds. Arrow on the *X*-axis indicates transfer to 16L:8D. An asterisk on the bar indicates the significance of difference at $p < 0.05$ level in mean values, as compared to the preceding observation.

3. RESULTS

The results are presented in Figures 1 and 2. At the beginning of the experiment, the absence of a significant difference ($F_{7,36} = 1.06$, $p = 0.4118$, 1-way ANOVA) in mean testis volume among various groups indicated total homogeneity in the reproductive status. Among control groups, both under long (14L:10D) and short (10L:14D) photoperiods, all birds underwent immediate testicular regression. In the 14L group the regression is clearly due to photorefractoriness, but in the 10L group it is unclear whether this is short-day induced regression, or a pre-programmed commitment to photorefractoriness and that 10L allows a recovery of photosensitivity. However, when these regressed birds were subjected to 16L:8D, only those exposed to 10L:14D showed testicular regrowth ($p < 0.05$, paired *t*-test; Fig. 1). This clearly indicated that birds exposed to 10L:14D were photosensitive when they were exposed to 16L:8D, and those maintained on 14L:10D had become refractory to a further long day photostimulation.

Among experimental groups too, all birds underwent testicular regression by the end of four weeks, irrespective of the light wavelengths they were exposed to (Figs. 2a–f), although there were small group differences in some light treatments; e.g., 3 of 5 birds receiving red light at zt 0–4 did not regress fully (Fig. 2c). That the experimental (spectral) groups show more gradual regression suggests that this is a short-day induced regression, with these groups interpreting photoperiod differentially as somewhere between 10L and 14L (Figs. 2a–f). When subjected to 16L:8D, testes were not restimulated in birds that were previously exposed to 14L:10D with a 4-h light period

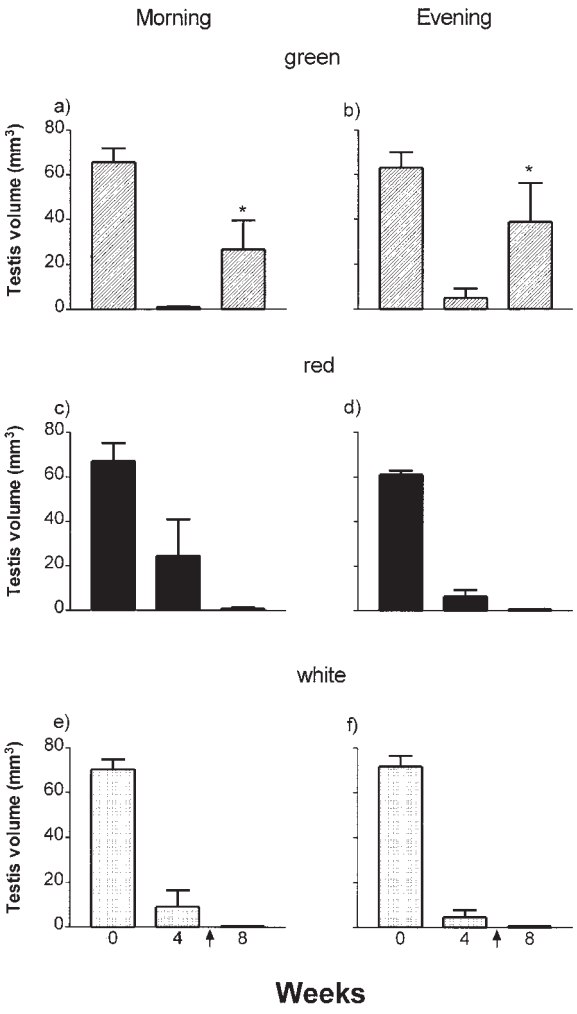

Figure 2. Maintenance of the photoperiodic responsiveness in blackheaded buntings under long day lengths of which a portion of light period was substituted with different light period. Gonadally stimulated birds were initially exposed to 14 hour light:10 hour darkness (14L:10D; L- 450 lux, D- 0 lux) for 3 weeks, and then a 4-h period in the morning or the evening of 14L was substituted with green (428 nm; **a, b**), red (654 nm; **c, d**) or white light (**e, f**) at 16 ± 2 lux intensity. After 4 weeks, indicated by an arrow on the *X*-axis, all birds were subjected to 16L:8D for another 4 weeks to test their sensitivity to long photoperiods as the consequence of exposure to spectral regimes. Note regrowth of testes only in groups previously exposed to 14L:10D with 4 h green light (a, b). Each bar represents the mean (± SE) testis volume for 5–6 birds. An asterisk on the bar indicates significance of difference at $P < 0.05$ level, as compared to the preceding observation.

in white or red colour (Figs. 2c–f), similar to 14L:10D controls. By contrast, testes recrudesced in birds that were previously exposed to 14L:10D with a 4-h light period in green colour, similar to 10L:14D controls (Fig. 2a and Fig. 2b). It appears that responsiveness to stimulatory effects of long day lengths was retained in birds previously exposed to 14L:10D with a 4-h period of green light (Fig. 2a and Fig. 2b), but not in birds previously exposed to 14L:10D with a 4-h period of white or red light (Figs. 2c–f). There was no difference in response to 16L:8D between the groups that received a 4-h different light period in the morning and that received it in the evening (cf. Figs. 2a–f; left and right panels).

4. DISCUSSION

A differential re-photostimulation of testes under 16L:8D, as the consequence of 4-week exposure to different spectral regimes (Fig. 2), suggests a wavelength-dependent effect on the photoperiodic

sensitivity: short, but not long, light wavelengths maintain the photosensitive state in the blackheaded bunting. Our result shows that the long day length of which a portion of light period contains wavelengths at 428 nm induces short day effects, but of which the same portion of light period contains wavelengths at 654 nm retains its long-day effects. This means that the perception by photoperiodic response system of the portion of 14L:10D at two light wavelengths is different. At 16 ± 2 lux intensity, a 428 nm light wavelength seems to be interpreted by buntings as dark period and, therefore, 14L:10D with a 4-h light period at this wavelength produced effects comparable to that of 10L:14D (cf. Figs. 1b and 2a, b). By contrast, at the same intensity, a 654 nm light wavelength seems to be interpreted as light period and, therefore, 14L:10D with a 4-h light period at this wavelength produced effects comparable to that of 14L:10D (cf. Figs. 1a and 2c, d). A similar situation with regard to the effects on avian photosexual response of light intensity is established. A long photoperiod at intensity lower than the threshold of perception by photoreceptors is interpreted as darkness and, hence, induces short day effects [4, 10, 11, 19, 22, 28, 29, 31].

A number of previous studies on this and other species support the role of light wavelength in avian photoperiodism (see especially [3, 5, 9, 14, 18, 22, 25, 26]). A most accepted explanation for the wavelength-dependent effects is that the number of photons received by the photoreceptors at different wavelengths is different: greater the number of photons available larger is the effect [32]. At equal energy level, the number of photons at long wavelengths is greater than at short wavelengths and, also, the penetration through tissues, hence access to brain photoreceptors, of long wavelengths is far more fast than of short wavelengths [2, 8, 23].

The photoperiodic effects of 14L:10D with a 4-h white light were similar to that of 14L:10D with a 4-h red light; both induced testicular regression and refractoriness (cf. Figs. 2c, d and 2e, f). Since white light contains both short and long light wavelengths (from 380 and 760 nm), the expectation was that it would produce an intermediate effect. The absence of an expected effect might mean that either long wavelengths overrode the effects, if any, of short wavelengths, or at 16 ± 2 lux intensity white light was interpreted as light period. A previous study shows that raising nighttime intensity to 10 lux under 12L:12D induces circadian and photoperiodic effects comparable to that are induced under continuous light, LL [16].

Our present data (Fig. 2) fail to show a phase-dependent effect of light wavelengths on the photoperiodic clock in blackheaded buntings, as suggested by other spectral studies [14, 25]. Except for individual variations in the rate and magnitude of gonadal regression in groups those received red light (e.g. Fig. 2c), there was no consistent difference between the morning and the evening groups (Fig. 2; cf. left and right hand panels). It seems that the interpretation by the photoperiodic response system of two light periods when given in sequence as one continuous light period, as in this study, is different than when they are given as two discrete light periods, as in previous studies [14, 25].

Given an identical structure of light regimes, the differential photoperiodic effects obtained in this study (cf. Figs. 1 and 2) were due to the difference in light wavelength and not due to the difference in light intensity. It appears, therefore, that under stimulatory long day lengths, the wavelengths of light influence induction of buntings from the photosensitive state into the photorefractory state. Thus, the current results, together with our earlier findings [e.g. 14, 25], suggest that the physiological processes involved in photostimulation and photorefractoriness in the blackheaded bunting are sensitive to changes in light wavelengths as they are to changes in day length.

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