CIRCADIAN RHYTHMICITY AND THE TERMINATION OF PHOTOREFRACTORINESS IN THE BLACK-HEADED BUNTING

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ABSTRACT.—Groups of photorefractory male Black-headed Buntings (*Emberiza melanocephala*), previously maintained on long daylengths (15L:9D), were exposed to photoperiods in which a 6-h light period was coupled with dark periods of 6, 18, 30, 42, or 54 h; a sixth control group was kept on 15L:9D. Cycles with lengths of 24 (6L:18D) and 48 h (6L:42D) terminated photorefractoriness, but cycles of 12 (6L:6D), 36 (6L:30D), and 60 h (6L:54D) did not; refractoriness persisted in the birds on 15L:9D. The results demonstrate that the termination and maintenance of the refractory period in Black-headed Buntings, a palearctic migratory passerine, are mediated by an endogenous circadian system of photoperiodic time measurement similar to that involved in the initiation and maintenance of gonadal growth, as is the case for several nearctic migratory species.

Endogenous rhythms with a period of approximately 24 h are involved in various photoperiodic responses of birds. For example, they are apparently responsible for the ability of short-day photoperiods to dissipate refractoriness in several nearctic species of birds from mid- and high latitudes (Hamner 1968, Murton et al. 1970, Turek 1972, Sansum and King 1975). In contrast, very little is known about the photoperiodic control systems of related *palearctic* species, e.g., those that migrate between the Indian subcontinent and southeast Asia. Our recent studies on one such species, the Black-headed Bunting (*Emberiza melanocephala*), reveal that it is photosensitive and that its photoperiodic responses are similar to those of many nearctic north temperate birds: long days cause gonadal growth, short days do not; and a period of insensitivity (refractoriness) to light, which develops in buntings that are photostimulated for long periods of time (Tewary and Kumar 1982, Kumar and Tewary 1983), is terminated by exposing them to short daylengths (Kumar and Tewary, unpubl.). In this paper, we report on resonance experiments that were designed to determine if a circadian rhythm of photosensitivity is involved in photoperiodic time measurement during the termination and maintenance of the photorefractory state of this palearctic emberizine, as it is in several nearctic relatives.

MATERIALS AND METHODS

The Black-headed Bunting is a sexually dimorphic migratory finch found in the subtropics. It arrives in Varanasi, India (25°18′N, 83°01′E), in the fall (September/October), overwinters, and then returns to breeding grounds in western Asia and eastern Europe (ca. 40°N) during the late spring (March/April; Ali and Ripley 1974).

Adult male birds were caught locally at Varanasi during fall 1980 and kept in an outdoor aviary. They were housed in small groups in wire-net cages (50 × 25 × 30 cm). In early December, groups were brought indoors and allowed to acclimate to laboratory conditions for 15 days. Here, they were exposed to natural variations of photoperiod (10.26 h of light daily) and temperature (ca. 22°C). Acclimated buntings were then put on a short daily photocycle consisting of 8 h light and 16 h dark (8L:16D; lights on beginning at 06:00) for eight weeks so that they would become photosensitive. Laparotomy at the end of this period showed that all birds had testes of minimal size (combined testicular weight [CTW] ≃ 5 mg).

These photosensitive birds were then photostimulated with 15 h light daily (15L:9D; lights on beginning at 06:00) for more than four months in order to induce testicular recrudescence followed by involution (photorefractoriness). (Exposure to 15L:9D for 120 days is adequate for such a testicular cycle in Black-headed Buntings; in contrast, exposure to 8L:16D for six months does not induce testicular development [Tewary and Kumar 1982].) The birds were laparotomized every four weeks during these months to determine testicular condition. On 29 June 1981, all of them had completely involuted testes (CTW ≃ 5 mg) and thus were presumably photorefractory. At this time, we divided them into one control

(*C*) and five experimental (G_1–G_5) groups of six birds each. We then exposed them to light-dark cycles of 15L:9D (*C*), 6L:6D (G_1), 6L:18D (G_2), 6L:30D (G_3), 6L:42D (G_4), and 6L:54D (G_5). Such light-dark cycles have been commonly used in testing the involvement of a circadian rhythm in photoperiodic responses of birds (reviewed by Farner 1975, Follett 1978).

Since an eight-week exposure to short day-lengths (8L:16D) appears to be enough to dissipate the refractory period in buntings (unpubl. observ.), all the birds were exposed to these resonance photoperiods for 60 days. Laparotomy at this time indicated that all of them had completely involuted testes. The birds were then reexposed to 15L:9D for 25 days, laparotomized again, and returned to the outdoor aviary.

Food and water were freely available, and were replenished only during the light phase of the daily photocycle. The cages were illuminated by fluorescent tubes that produced an intensity of about 300 lux at perch level. The temperature of the photoperiodic chambers was not closely regulated, but it did not vary more than 2°C from the temperature of the laboratory. (During the approximately nine months of our study, the temperature of the laboratory varied from about 22°C in December to 35–38 and 30–32°C in June and the following September, respectively.) The CTW of each bird was estimated visually by comparing the size of its testes in situ with a reference set of fixed gonads of known weight. The error inherent in this method is about 20%. Statistical comparisons between the control and experimental groups of buntings were done with Student's *t*-tests.

RESULTS

Figure 1 demonstrates the effectiveness of various light-dark cycles in dissipating photorefractoriness in Black-headed Buntings. Birds maintained on 15L:9D had regressed testes throughout the second part of the experiment, i.e., remained photorefractory. Those previously exposed to cycles of 24 (6L:18D) and 48 h (6L:42D), but not those exposed to 12 (6L:6D), 36 (6L:30D), and 60 h (6L:54D), showed testicular enlargement ($P < 0.001$) after their return to 15L:9D. Thus, 12-, 36-, and 60-h cycles acted like long days (maintained the refractory state), whereas 24- and 48- cycles acted like short days (terminated the refractory state), even though each experimental photoperiod contained only a 6-h light phase.

Eight birds, one each from *C*, G_2, G_4, and G_5, and two each from G_1 and G_3, died during
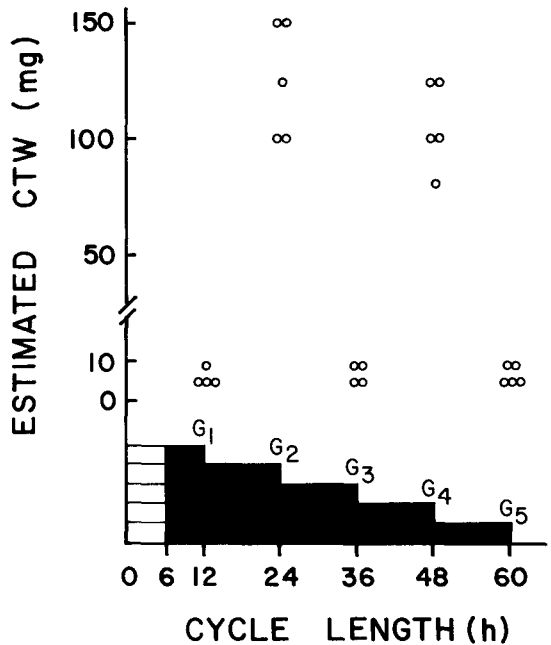

FIGURE 1. The effectiveness of various light cycles in terminating the refractory period of Black-headed Buntings. The closed bars represent durations of the dark phase following the 6-h light phase (open bar) in each light/dark cycle. The symbols denote the combined testicular weight (CTW) of individual birds in each group (G_1 – G_5). A control group, the data for which are not presented in the figure, was kept on 15L:9D. The mean estimated CTW of the control buntings was not statistically different from that of G_1, G_3, and G_5. However, the testes of buntings in G_2 and G_4 enlarged significantly ($P < 0.001$; with reference to the size of testes in the control group) when the birds were subsequently exposed to 15L:9D.

the experiment. No data are presented for them in Figure 1.

DISCUSSION

Our results are consistent with the Bünning (1973) hypothesis and indicate that an endogenous rhythm with a period of approximately 24 h is involved in photoperiodic time measurement of photorefractory Black-headed Buntings. In general, the data in Figure 1 are in agreement with those of Turek (1972) on photorefractory White- and Golden-crowned sparrows (*Zonotrichia leucophrys* and *Z. atricapilla,* respectively), i.e., nearctic passerines. However, an 8L:8D cycle terminated the refractory period of White-crowned Sparrows, but not that of Golden-crowned Sparrows. (This inconsistency between the species, however, simply means that their endogenous rhythms are entrained by 8L:8D in different manners.)

Hamner (1968) and Murton et al. (1970) had suggested earlier that a circadian rhythm is involved in the termination of photorefractoriness, but their experiments were not con-

clusive. However, Sansum and King (1975) demonstrated in Gambel's White-crowned Sparrows (*Z. l. gambelii*) that such a rhythm was indeed involved in ending the refractory state. A similar rhythm has recently been discovered in the palearctic Red-headed Bunting (*Emberiza bruniceps*; Prasad and Tewary 1983).

Endogenous circadian rhythms, probably of multiple phylogenetic origin, appear to be involved in time measurement in the photoperiodic responses of a number of avian species (see reviews by Farner 1975, Follett 1978, Turek and Campbell 1979). Comparative studies to date, however, including one on Black-headed Buntings (Tewary and Kumar 1981), have dealt with the initiation or maintenance of gonadal growth, rather than photorefractoriness (Hamner 1963, Farner 1965, Follett and Sharp 1969, Turek 1974, Gwinner and Eriksson 1977).

In Black-headed Buntings, the initiation of testicular growth depends on long days, and the termination of photorefractoriness on short days. Although other models can explain how a circadian rhythm is involved in these two photoperiodic responses, our data for both the initiation of gonadal growth and the termination of the refractory period are consistent with an external coincidence model for photoperiodic time measurement. According to this model, a physiological response results when light is present during a particular (photosensitive) phase of a circadian rhythm. Apparently, photorefractoriness is maintained when light is coincident with the photosensitive phase; but is dissipated in the absence of such coincidence. Under the latter condition, photoperiodically-induced gonadal growth can reoccur when birds are subsequently exposed to long days.

We do not know if the factor(s) that caused the high mortality in the present study also affected the gonadal responses of the buntings that survived. Although Black-headed Buntings do not often die when kept in captivity for short periods, they do succumb when kept for periods of more than six to seven months. In our experience, the incidence of mortality is especially high in males at the time when they become photorefractory. High mortality also occurred in Red-headed Buntings during studies similar to those described here (Prasad and Tewary 1983).

ACKNOWLEDGMENTS

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