Circadian Involvement in Termination of the Refractory Period in Two Sparrows

Abstract. *Endogenous daily rhythmicity is involved in the short-day photoperiodic response of breaking the refractory period in both white-crowned and golden-crowned sparrows. Under a modified "coincidence model," short-day photoperiodic induction occurs when light is noncoincident with a specific phase of an internal rhythm.*

In 1936 Bünning proposed that an organism's endogenous daily rhythm may be causally related to measurement of photoperiodic time (*1*). In his hypothesis, the endogenous circadian rhythm consists of two half cycles called the photophilic and scotophilic phases; the photoperiodic effect of a particular light-dark cycle is dependent on which portion of the underlying circadian rhythm is illuminated. In an organism in which longer periods of daylight result in a photoperiodic response, the short days of winter restrict the illumination of light to only the photophilic phase of the circadian rhythm. As the days become longer, light is extended into the second half of the innate rhythm (the scotophilic phase), and photoperiodic induction occurs.

A more explicit version of Bünning's hypothesis was advanced by Pittendrigh and Minis (*2*), who stressed the dual role of light as both the entraining agent of the circadian rhythm and the inducer of the photoperiodic response. In their "coincidence model," photoperiodic induction occurs when light is coincident with a specific phase of the circadian rhythm, termed the photoperiodic inducible phase. This inducible phase may not necessarily last a full 12 hours, as does Bünning's scotophilic phase.

A relation between the circadian rhythm and a photoperiodic response has been demonstrated in several species of birds (*3–5*). These experiments have generally been confined to the initiation or maintenance of gonadal growth. Hamner (*6*) and Murton *et al.* (*7*) raised the possibility that a circadian clock may be involved in measuring the photoperiod during the refractory period, but their experiments were not conclusive. The research reported here was undertaken to determine if a circadian rhythm of response to light is involved in the termination of the refractory period in the white-crowned sparrow (*Zonotrichia leucophrys pugetensis*) and the golden-crowned sparrow (*Z. atricapilla*).

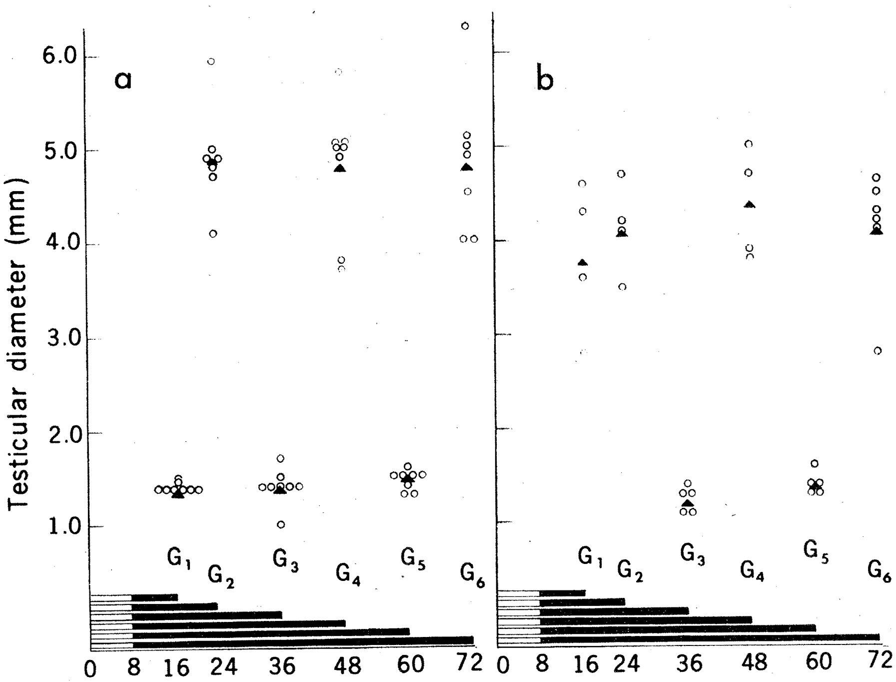

Fig. 1. Data on the diameter of the left testis for golden-crowned sparrows (a) and white-crowned sparrows (b). The solid bars represent the different durations of darkness following the standard 8-hour light period. All birds were first exposed to these light cycles for 6 weeks. They were then transferred to an LD 16 : 8 light cycle for 6 weeks. Shown is the maximum testicular diameter after 4 to 6 weeks of the LD 16 : 8 cycle; ○, individual birds in each group; △, the mean for each group; *G*, group.

After a period of gonadal activity, the reproductive organs of birds regress and the breeding season is terminated. In most temperate zone birds this regression often occurs in midsummer, even though the period of daylight is as long as or longer than that which initiated gonadal growth in the previous spring. After regression is complete, the birds enter a state of gonadal inactivity called photoperiodic refractoriness. Regardless of the light regime, gonadal growth cannot be induced in birds in the refractory condition (*8*). If white-crowned and golden-crowned sparrows are exposed during the refractory period to long days—such as 16 hours of light followed by 8 hours of darkness (LD 16 : 8)—they will remain in the refractory state even through the following spring (*9*). However, if the birds are exposed to short days for 6 weeks, the refractory period will be terminated, and a light-dark cycle of LD 16 : 8 will again be able to induce gonadal growth (*10*). In nature such short exposure to daylight occurs in the fall.

Male white-crowned and golden-crowned sparrows were captured with mist nets and standby traps in late winter of 1971 near Stanford University. All birds were maintained in outdoor aviaries until the beginning of experimental treatment, when they were placed in indoor cages of various sizes. In all indoor chambers the light intensity was greater than 40 lux, which is well above the photoperiodic threshold for these two species (*11*). The width of the left testis (small diameter to the nearest 0.1 mm) was measured in situ following unilateral laparotomy.

Throughout the spring and early summer, birds in the outdoor aviary were periodically examined to determine the reproductive condition of their testes. On 29 and 30 July 1971, all birds examined by laparotomy (48 *Z. atricapilla* and 34 *Z. leucophrys pugetensis*) were in a state of complete testicular regression and thus were in the refractory period of their testicular cycle (*12*). On 1 August the birds were brought into the laboratory and divided evenly into six groups and placed on the following light schedules: group 1, LD 8 : 8; group 2, LD 8 : 16; group 3, LD 8 : 28; group 4, LD 8 : 40; group 5, LD 8 : 52; and group 6, LD 8 : 64.

With such light cycles one can detect recurring phases of responsiveness to light about 24 hours apart (*13*). Two golden-crowned and six white-crowned sparrows died during the experiment.

I found previously (*10*) that 6 weeks of short days (LD 8 : 16) were enough to terminate the refractory period in both species. Therefore, after 6 weeks of exposure to the experimental light cycles, all birds were transferred to an LD 16 : 8 cycle to determine in which groups the refractory period had been broken. At the time of transfer, measurements of testicular size indicated that all birds were still in a state of complete testicular regression.

The effectiveness of the various light cycles in breaking the refractory period is shown in Fig. 1. Maximum testicular increase after 4 or 6 weeks of the LD 16 : 8 cycle is plotted for the various preceding light cycles. Testicular enlargement occurred in all golden-crowned sparrows (Fig. 1a) previously subjected to cycles of 24 hours (LD 8 : 16), 48 hours (LD 8 : 40), and 72 hours (LD 8 : 64). No enlargement occurred in golden-crowned sparrows subjected to cycles of 16 hours (LD 8 : 8), 36 hours (LD 8 : 28), or 60 hours (LD 8 : 52). The 24-, 48-, and 72-hour cycles all had the effect of short days; these cycles terminated the refractory period and resulted in photosensitivity to long days (LD 16 : 8). On the other hand, 16-, 36-, and 60-hour cycles maintained the refractory state. These cycles acted as long days, even though each cycle contained only one 8-hour period of light.

These results are consistent with the hypothesis that an endogenous rhythm with a periodicity of about 24 hours is used in measuring the length of the photoperiod during the refractory state of the golden-crowned sparrow. Apparently, photoperiodic refractoriness is maintained when light is coincident with a certain phase of the circadian rhythm. When light is noncoincident with this phase, the refractory period is broken, and testicular growth can again be initiated by long days.

A circadian rhythm is also involved in the termination of the refractory period in the white-crowned sparrow (Fig. 1b). These results differ only in that the 16-hour cycle (LD 8 : 8) did terminate the refractory period in white-crowned sparrows, but not in golden-crowned sparrows. The response of white-crowned sparrows to the 16-hour cycle is not understood, but this result does not negate the hypothesis that a circadian rhythm is involved in this photoperiodic response. The results from the other light cycles indicate that an endogenous rhythm with a periodicity of about 24 hours is involved in breaking the refractory period of the white-crowned sparrow. The peculiar results for the 16-hour cycle reemphasize the importance of understanding the dual action of light, both as an entraining agent of the endogenous rhythm and as the inducer of the photoperiodic response. The phases of the circadian rhythm which are coincident with the light in the 16-hour cycle will be different from the phases which are coincident with the light in the 36- and 60-hour cycles. Without an understanding of how an LD 8 : 8 cycle entrains the circadian rhythm involved in this photoperiodic response, one cannot determine why such a cycle ended the refractory period in white-crowned sparrows.

Farner (*4*) found that an endogenous rhythm may be involved in the initiation of testicular growth in *Z. leucophrys gambelii*. In an experiment similar to that done by Hamner on the house finch *Carpodacus mexicanus* (*3*), I found that circadian rhythmicity is involved in the initiation of testicular growth in both *Z. leucophrys pugetensis* and *Z. atricapilla* (*14*).

In both the white-crowned and golden-crowned sparrows the initiation of testicular growth is normally dependent on long days, whereas the termination of the refractory period depends upon short days. While there exist other possible models for how the circadian clock may be involved in these two photoperiodic responses (*15*), I believe the "external coincidence model" is at present the most likely possibility. Under this hypothesis, the initiation of testicular growth depends on the coincidence of light and a particular phase of the circadian rhythm. The termination of the refractory period depends on the lack of coincidence of light and a particular phase of a circadian rhythm.

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References and Notes

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13. One of the most conclusive methods in determining if an endogenous rhythm is involved in a photoperiodic response is to couple a fixed light period to a variety of different dark periods. The rationale for using such light cycles in testing for the involvement of a circadian clock in a photoperiodic response is given in (*3*).
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16. I thank C. S. Pittendrigh for comments and criticism and for his support through the Whitehall Foundation, E. Gwinner for many valuable discussions, and P. Turek and P. Caldarola for technical assistance in the preparation of the manuscript.

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Summated Cortical Evoked Response Testing in the Deafferented Primate

Abstract. *Dorsal rhizotomy from C_2 or C_3 to T_4 in the primate results in failure to elicit summated cortical responses from systematic stimulation of the appropriate peripheral nerves. Under these conditions there is thus no evidence of sensory input into the cerebral cortex. A nonclassical mechanism must therefore be operational to explain the extensive purposive movements observed in the deafferented animals.*

Deafferentation of a single forelimb in rhesus monkeys by means of dorsal root section has long been known to result in an effectively useless extremity when the animal is unrestricted (*1*). However, a monkey can be induced to use the affected limb purposively by increasing the motivation to do so, as in conditioned response situations where reinforcement is made contingent on use of the deafferented member, or by subjecting the contralateral limb to prolonged restraint (*2*).

The ability to make purposive movements following the abolition of somatic sensation and autogenetic spinal