# *EXTRARETINAL LIGHT PERCEPTION IN THE SPARROW, I. ENTRAINMENT OF THE BIOLOGICAL CLOCK**

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A small but convincing body of literature indicates the existence of photoreception by nervous tissue which is not obviously specialized for this function. Among the invertebrates, perhaps the most thoroughly studied case is the caudal ganglion of the crayfish, which possesses two neurons that respond electrically to light stimuli.[1] The pineal organs of fish, amphibians, and reptiles, though not of birds and mammals, have been shown to be light-sensitive.[2] Sunlight penetrates to the hypothalamus of several mammalian species;[3] however, the only indication that light may exert a physiological effect directly on the mammalian brain comes from the work of Lisk and Kannwischer,[4] who showed that selective illumination of several areas of the hypothalamus affected the estrous cycle in blinded rats. An elegant series of studies by Benoit and co-workers[5] has established that testis growth, a system under partial photoperiodic control, can be effected by light impinging directly on the hypothalamic area of the brains of blinded ducks. Benoit's results have not stimulated other workers to perform similar experiments, probably because of the great difficulty encountered in keeping blinded birds of most species alive, and remain the only published evidence for light sensitivity of the avian brain.[6]

Circadian rhythms of locomotor activity have been extensively studied in many species of passerine birds[7] as well as in rodents, insects, and a variety of other animals. In constant darkness or constant dim light, such rhythms universally exhibit free-running periods, characteristic of both the species and the individual animals studied and generally different from 24 hours. Without exception, free-running rhythms can be entrained (synchronized) by exposing the organism to light-dark cycles with periods of exactly 24 hours. The entrained rhythm has a period of exactly 24 hours as well as a determinate phase relationship to the light cycle. Entrainment to a light cycle can thus serve as a definitive and easily obtainable assay of light perception.

Several workers have studied circadian rhythms of blinded mammals in the presence of light cycles and have reported that the rhythms were free-running, as if the animals were in constant darkness.[8] It therefore appears that in mammals retinal light perception may provide the only route by which information about the environmental light regimen reaches the centers that control circadian rhythms. There are no previous reports of experiments with circadian rhythms of blinded birds. The present paper reports on experiments which demonstrate that the house sparrow (*Passer domesticus*) possesses an extraretinal photoreceptor(s) which is coupled to the "clock" system controlling the overt circadian rhythm of locomotor activity. The sensitivity of this receptor and the response of the free-running period of the rhythm of blinded birds to constant light of various intensities are also discussed.

*Materials and Methods.*—Field-caught sparrows of both sexes were maintained in individual cages in lightproof wooden boxes and their activity was recorded as previously reported.[9] Two light sources, each controlled by a clock, were installed in each box: a 4-watt "cool white" fluorescent bulb (Ken Rad F4T5/cw) which produced an intensity of about 500 lux at the level of the perches and a 0.02-watt electroluminescent panel (Sylvania "Panelescent Nite-Lite") which produced about 0.1 lux of green light at perch level.

Birds to be enucleated were anesthetized by intramuscular injection of Equithesin (Jen-Sal). The eyeball was first freed from the lids and the muscles were severed with an iridectomy scissors working from the dorsal to the ventral surface. Finally the optic nerve was cut and the eyeball was removed. Puncturing the posterior chamber and allowing it to drain simplified the surgery. Fairly severe bleeding was often encountered. The empty socket was wiped with gauze soaked in 70% ethanol and packed with absorbable gelatin sponge (Gelfoam-Upjohn). After one eye had been removed, the bird was held in an activity cage for 3–5 days during which it received tetracycline hydrochloride (Polyotic-American Cyanamid) in its drinking water. During this time the bird recovered from the surgery and in addition learned the position of the feeding and water jars and the two perches. The second eye was then removed following the same procedure. Sparrows appear to tolerate bilateral enucleation well. Less than 5% died as a direct result of the operation. As long as the geometry of the feeding and watering jars and of the perches was not disturbed, bilaterally enucleated sparrows had no difficulty eating, drinking, or hopping from the floor of the cage to the perches. Some birds appear healthy more than a year after bilateral enucleation and very few have died in the course of our experiments.

The perching behavior of each bird was continuously recorded on one channel of a 20-channel operations recorder. The chart was cut at 24-hr intervals (18 inches) and each day's record was pasted under the record of the preceding day. The figures are photographic reductions of the raw data handled in this manner.

*Results.*—(a) *The extraretinal perception of light:* Figure 1 is the perching record of a bilaterally enucleated sparrow. For the first six days of the experiment the bird was in constant darkness (DD), and its perching rhythm had a circadian period of about 24 hours 20 minutes as judged by the onsets of activity. On day 7 a light cycle was presented to the bird (LD *12*:12, 500 lux:0 lux) as indicated in the figure. After three to four days of transients the period of the locomotor rhythm became the same as that of the light cycle (24 hr), and the phase of the activity onset was about 10–15 minutes before the daily onset of light. On day 40 the light cycle was changed to LD *18*:6 (500:0) by the addition of six hours of light at the end of the previous light period. This change in the lighting regimen produced two changes in the bird's perching behavior. The activity onset took up a new steady-state phase relationship with the onset of light. After four days of gradually delaying transients, the bird's activity began about 50 minutes after the daily onset of light. In addition, the amount of time each day for which the bird was active increased from about 12 hours to approximately 17 hours. On days 78 and 79 two power failures occurred in the laboratory. These had the net effect of delaying the light cycle by 9.5 hours. From day 79 on, the light came on at 1:30 A.M. instead of 4:00 P.M. The bird shifted his activity to follow the newly phased light cycle almost immediately. However, the steady-state phase of the activity onset now preceded the onset of light by about 40 minutes. On day 117 the bird was returned to constant darkness and expressed a circadian free-running rhythm with a period of about 25 hours.

We have examined the response of 53 bilaterally enucleated sparrows to 24-hour light cycles of several different photoperiods at 500 lux. Without exception the birds show entrainment to these cycles. The phase of the activity rhythm is always correlated with the light cycle in the particular box in which the bird is held and not with either the external day or the light, temperature, or noise cycle in the laboratory. Therefore, attempts to determine the nature of the stimulus to which enucleated birds respond can be narrowed to those factors associated with the box light cycle.

Several kinds of stimuli suggest themselves. There is, of course, a temperature cycle produced in the box by the fluorescent bulb itself (the ballast transformer

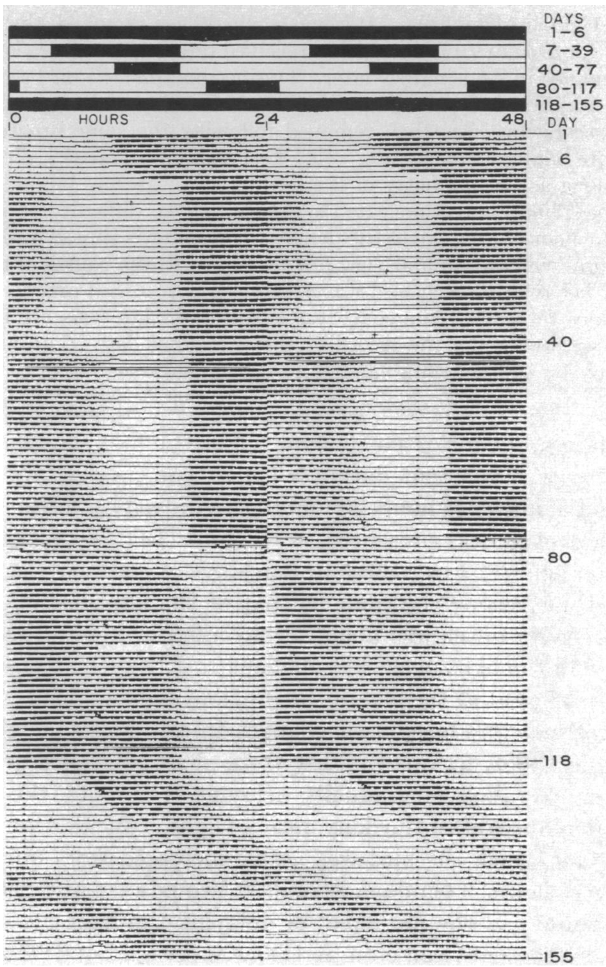


FIG. 1.—Continuous record (155 days) of perching activity of a blinded sparrow. The several light regimens (500 lux) are shown diagrammatically in the upper portion of the figure. Note that when a light cycle is present it controls the phase and period of the activity as well as the duration of activity per day (compare LD *12*:12 with LD *18*:6). The record has been duplicated with the right-hand portion displaced upward one day to aid in visual inspection. Hour 0 = midnight Central Standard Time. Blank spaces are due to occasional pen failure.

is mounted on the outside of the box). The amplitude of this cycle is about 1.5°C, which is considerably less than the 5°C temperature cycle which often occurs in the box as a result of daily variation in the temperature of the entire laboratory. If the birds were responding to temperature, the phase of the rhythm would be expected to correspond with the laboratory cycle rather than with the cycle produced by the bulb. Attempts to demonstrate entrainment by temperature cycles with hamsters[10] and with one species of bird, the house finch,[11] have been unsuccessful. In the latter experiments, temperature cycles with amplitudes of 20°C did not entrain, although small effects on the free-running period were observed. Of course, none of the above arguments completely rules out the possibility that enucleated sparrows may be entrained by a temperature cycle of low amplitude.

Figure 2 is the activity record of a bilaterally enucleated sparrow in DD until day 15 when it was presented with an LD *12*:12 cycle using only the electroluminescent panel as a light source. Four days of transients are followed by steady-state entrainment to the signal in which the phase of the onset of activity occurs about an hour after the daily onset of light. In contrast to their response to the 500-lux white light signal, not all enucleated birds entrain to 12 hours of 0.1-lux green light. Of the 22 blind birds presented with LD *12*:12 (0.1 green: 0) 12 entrained, while the other 10 free-ran as if in constant darkness. All normal birds entrain to this stimulus. The fact that some blind birds clearly entrain to the electroluminescent panel strengthens the argument against temperature as the entraining stimulus. We were unable to measure an increase in the box temperature due to the presence of the lighted panel. The electroluminescent panel emits virtually no infrared energy and thus the possibility that sparrows possess special infrared receptors responsible for entrainment can be provisionally discarded.

Several experiments were performed with bulbs heavily wrapped with black tape in order to definitively eliminate possible entraining effects of temperature changes, infrared radiation, the slight vibration produced by the ballast transformer, the low hum produced by some fluorescent bulbs, as well as any other variables associated with the light cycle, except for visible light itself. Figure 3 is the record of a bird free-running in the presence of a "light" cycle produced by a 4-watt fluorescent bulb wrapped with several layers of black electrical tape. On day 34 the wrapped bulb was replaced with a normal one and the bird entrained to the now visible light cycle.

The possibility that small islands of functional retina remain after enucleation seems unlikely in view of the facts that (1) eyes were microscopically examined for torn retinae upon removal and very few incomplete retinae were found, and (2) birds continue to entrain to light cycles 10 months after enucleation, by which time any remaining retinal structure must have degenerated.

Enucleated birds treated with Dry-Die[12] to remove external parasites also entrained to light cycles. While it would be difficult to be certain that all parasites had been removed from any particular bird, this method has been shown to be extremely effective.[13] The general similarity of the response to light cycles in blind and normal birds, and the response of blind birds to constant light of

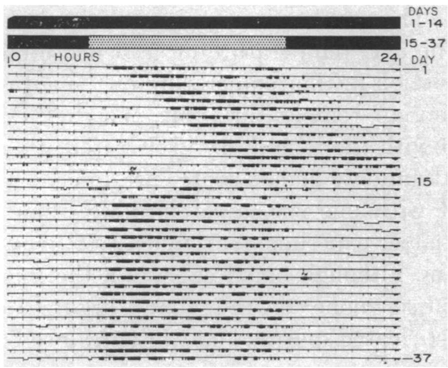


FIG. 2.—Entrainment of a blinded sparrow to a light cycle in which the light (indicated by stippling in diagram) is approximately 0.1 lux (green light). During entrainment the onset of intense activity occurs 30–60 min after the light comes on.

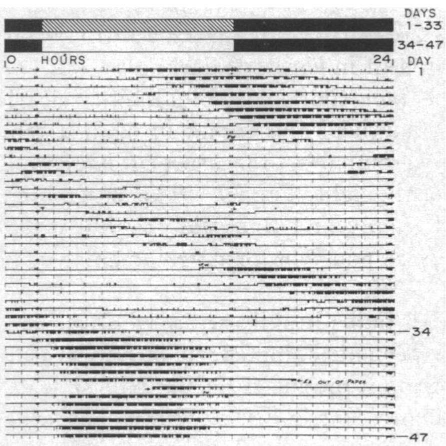


FIG. 3.—Perching record of a blinded sparrow free-running through a "light" cycle (shaded area in diagram) produced by a 4-watt fluorescent bulb heavily wrapped in black tape. On day 34 this bulb was replaced with an unwrapped one, producing a light cycle to which the bird subsequently entrained. The sporadic activity recorded on days 10–25 resulted from a faulty microswitch which was repaired on day 26.

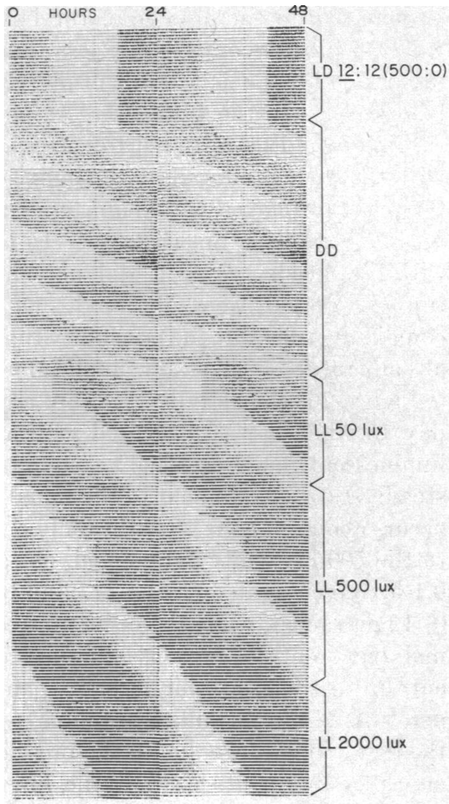


FIG. 4.—"Aschoff's rule" in a blinded sparrow. The continuous record of perching activity (251 days) has been duplicated as described in Fig. 1. Note the large changes in free-running period and in the proportion of "activity time" to "rest time" when the bird goes from constant darkness to constant light of 50 lux. Upon raising the light intensity to 500 lux small changes in both of these parameters occur as well as what is probably a phase shift requiring approximately 35 days of transient cycles for completion. Further increase to 2000 lux produces little change, if any. (The shaded area with a precise 24-hr period, which begins several days before the end of the DD treatment, is an artifact produced by the adjacent channel of the operations recorder.)

different intensities (see below) render remote the possibility that enucleated birds perceive the light *via* the behavior of their ectoparasites.

Taken together, the above experiments argue very strongly in favor of the hypothesis that house sparrows possess an extraretinal photoreceptor(s) which is coupled to the circadian clock controlling locomotor activity.

(b) *Properties of the entrainment response:* The entrainment of enucleated sparrows to light cycles is remarkably similar to that of normal birds. The steady-state phase angle to LD *12*:12 (500:0) is about the same in both groups of birds, but the negative phase angles seen in some blind birds on longer photoperiods are never observed in normal birds at this light intensity. The number of "transient" cycles required to reach steady state may be greater in blind than in normal birds, although data for a comparison of this response before and after enucleation in the same bird are not presently available. Judging from the experiments in which the electroluminescent panel is used as the only light source, the light intensity threshold for entrainment is higher in blind than in normal birds.

(c) *Properties of the free-running rhythms:* No obvious and consistent differences between the free-running rhythms of blind and normal birds in constant darkness have appeared in the data. Blinded birds obey "Aschoff's rule," an empirical generalization which describes the response of most circadian rhythms to light intensity under constant conditions.[14] According to Aschoff's rule, the free-running period of diurnal organisms shortens as the light intensity increases and the ratio of activity time to rest time within each cycle increases ($\alpha/\rho$ ratio). The response of a blind sparrow to constant light of different intensities is shown in Figure 4.

There is one important difference between blind and normal birds in their response to constant light. All normal sparrows which we have recorded in constant light become arhythmic (i.e., they are active almost continuously and no circadian pattern is evident in their perching records) in constant light of 500 lux and above. However, as can be seen from Figure 4, blind birds do not become arhythmic at 500 lux or in fact at 2000 lux. Further, there is very little change in the free-running period or the $\alpha/\rho$ ratio as a result of raising the intensity of the constant light from 500 to 2000 lux. The mechanism underlying the response known as Aschoff's rule has become saturated at some intensity below 500 lux without the arhythmicity usually produced in circadian systems by high levels of constant light.

*Discussion.*—On the basis of Benoit's work and the fact that the bird skull is thin and translucent, one is tempted to assume that the extraretinal photoreceptor (referred to below as ERR) is located in the brain. The hypothalamic area[4, 5] and/or the pineal gland seem, *a priori*, reasonable sites for further investigation. However, preliminary experiments[15] indicate that the presence of the pineal gland is not necessary for the entrainment response of blinded sparrows and with the data at hand we are not able to rule out the possibility that the skin or some other tissue is mediating the response to light.

Some differences do exist between the responses to light of blinded and normal sparrows. The negative phase angles of enucleated birds to long photoperiods, their greater number of transient cycles during the approach to the entrained steady state, the maintenance of rhythmicity at high light intensities, and the failure of about 50 per cent of blinded sparrows to entrain to the very low intensity produced by the panelescent light can all be most simply explained by the assumption that removal of the eyes decreases the effective intensity of the light stimulus. However, note that this interpretation does not necessarily imply that the retina, when present, is functionally involved (see below).

One of the most interesting questions raised by the finding that enucleated sparrows entrain to light cycles is: What are the relative contributions of the eye and the ERR to entrainment in the intact bird under normal light conditions? This question can only be answered by further investigation of the similarities and differences in the responses of blind and normal birds, but it seems worthwhile to take stock of the position into which the presently available data lead us.

In the presence of eyes, the ERR may be without effect on entrainment. The similarities of the entrainment response in blind and normal birds, which far outweigh the differences, argue against this possibility.

The ERR may provide the only route by which light cycles are able to influence the circadian clock. If this were the case, then the predicted effect of enucleation on the entrainment response would depend on the path by which light normally reached the ERR. If this path were through the skull and brain tissue only, then enucleation would be expected to be without effect. However, if, as seems possible, the eyes provide "windows" through which light normally reaches the ERR, then their removal could either increase the effective intensity of a given signal by removing structures which impede the passage of light, or decrease it through the formation of scar tissue which might render the window more opaque. In this connection it should be emphasized that the electroluminescent panels emit chiefly green light, which does not penetrate tissue nearly as well as do longer wavelengths. The reduced response of enucleated birds to dim green light could result from a decrease in effective intensity at these wavelengths due to the formation of scar tissue in the eye sockets.

Perhaps the most reasonable hypothesis is that both the retinae and the ERR contribute to the entrainment response. However, there are at present no data which demand the conclusion that retinal light perception is ever involved, whereas light perception by the ERR clearly plays a role under certain circumstances.

Referring both to photoperiodic control of reproductive cycles and to the entrainment of circadian rhythms, Kennedy in 1960[16] said, "One might . . . be cautious about assuming that . . . control is mediated by 'obvious' photoreceptor structures in any given case." His caution would appear to be amply justified.

*Summary.*—(1) House sparrows (*Passer domesticus*) can be blinded by bilateral optic enucleation. They survive this operation well and have subsequently been maintained, free-living in cages, for up to 10 months. No other species of bird has been reported to survive blinding under these conditions. The sparrow is clearly the avian species of choice for experiments in which the pathways by means of which light exerts its physiological effects are of interest.

(2) The circadian activity rhythms of blinded sparrows entrain to (are synchronized by) 24-hour cycles of visible light alternating with darkness and do not entrain to the cycles of temperature, noise, infrared radiation, and vibration which are associated with the light cycle. The sparrow must therefore possess an extraretinal photoreceptor which is coupled to its biological clock.

(3) The extraretinal photoreceptor is surprisingly sensitive. About 50 per cent of blinded sparrows entrain to cycles in which the light portion consists of green light of 0.1 lux. However, by several criteria, the clocks of blinded birds do not appear to be as sensitive to light as those of normal birds.

(4) Blinded sparrows obey "Aschoff's rule." In constant light, the free-running period shortens and the ratio of activity time to rest time increases as the light intensity is raised.

(5) In general the response of the locomotor rhythm of blinded birds to light cycles and to constant light of different intensities is remarkably similar to that of normal, sighted birds. The small differences which do exist can be accounted for on the assumption that blinding reduces the effective intensity of the light stimulus.

(6) The possible anatomical location of the extraretinal photoreceptor and its role, as well as that of the retinae, in the normal entrainment response of sighted birds are discussed.

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