On the Role of Eyes and Brain Photoreceptors in the Sparrow: Aschoff's Rule

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Summary. The period (τ) of the House Sparrow (*Passer domesticus*) free-running activity rhythm obeys "Aschoff's Rule", shortening when the level of constant illumination is increased and lengthening when illumination is decreased (Fig. 1). This response is mediated by both the eyes (Figs. 2 and 3) and the extraretinal brain photoreceptors (Figs. 3, 4 and 5, Table 1).

Introduction

The circadian locomotor activity rhythm of the House Sparrow, *Passer domesticus*, free-runs in constant darkness (DD) or constant dim light (LL). The rhythm exhibits a free-running period (τ) the length of which is almost always different from 24 hours and is influenced by the level of illumination. In most diurnal vertebrates τ shortens as the light intensity is increased and lengthens as the light intensity is reduced. Sparrows, for example, express τ's of approximately 25 hours in DD and of less than 24 hours under certain intensities of LL (see Fig. 1). This characteristic response of τ to light intensity has been termed "Aschoff's Rule" (Hoffmann, 1965).

Blind (eyeless) sparrows obey "Aschoff's Rule" (Menaker, 1968). Sparrows possess extraretinal brain photoreceptor(s) (ERP) which are coupled to the circadian clock timing locomotor activity (see Menaker, 1971). The eyes also participate in some of the effects of environmental lighting on the clock (McMillan, Keatts and Menaker, 1975; McMillan, Elliott and Menaker, 1975). We here report experiments which demonstrate that the response of τ to the intensity of constant light is mediated by both the eyes and the ERP.

Materials and Methods

House Sparrows were collected, maintained, blinded, head feather plucked, and carbon black was injected or removed as described previously (McMillan, Keatts and Menaker, 1975). The birds were caged in individual light-tight enclosures and perch-hopping was recorded continuously (Menaker, 1965). Biweekly feeding and cage cleaning were conducted so that conditions of constant darkness or dim light were not altered.

Three sources provided constant light; Sylvania "Panelescent Nite-Lites" (approximately 0.1 lux, green light), 4-Watt "cool white" fluorescent bulbs (Ken Rad F4T5/cw), and $7^{1}/_{2}$-watt incandescent bulbs (General Electric). The intensity of the fluorescent bulbs was adjusted by masking with black plastic tape; incandescent bulbs were partially sprayed with flat black paint. Unless otherwise indicated, experiments employing incandescent light were repeated with fluorescent light of the same intensity and qualitatively identical results were obtained. Light measurements were made at perch level.

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Hooding consisted of carbon black injection beneath the skin of the head followed by painting of the head with collodion saturated with Sudan Black B (see McMillan *et al.*, 1975). Hood removal involved cutting away the blackened collodion, a skin incision and scraping of the carbon black deposit.

Results and Discussion

Normal House Sparrows obey "Aschoff's Rule"; the period length (τ) of the perch-hopping activity rhythm decreases with increasing levels of constant illumination (Fig. 1). Treatments altering the photic input to the circadian clock via the eyes (blinding) or the ERP (hooding, carbon black injection, head feather plucking, hood or carbon black removal) of birds free-running in constant light cause stable changes in τ which are predicted by "Aschoff's Rule" coupled with the assumption that the circadian system sums the effects of light reaching the clock by the two routes (Table 1). Blinding produces a lengthening of τ (Figs. 2 and 3). Hooding, which decreases the light reaching the brain by a factor of between 100 and 1,000, causes a lengthening of τ in blind birds (Fig. 3). Removal of the hood results in a shortening of τ (Fig. 4). Head feather plucking, which increases the amount of light reaching the brain 10 to 100 fold, shortens τ in both blind and sighted birds. Carbon black injection beneath the skin of the head reduces light to the brain 10 to 100 fold and results in a lengthening of τ (Fig. 5). Control birds injected with saline solution show no change. Our results demonstrate that both the eyes and the extraretinal photoreceptors of sparrows can act as intensity discriminators mediating graded changes in free-running period as a function of changes in light intensity.

It is worth remarking on the two different effects on free-running period which we have observed following removal of the first eye (c.f. Figs. 2 and 3). In about 50% of our experiments this operation resulted in a persistent lengthening of τ and little or no further change was produced by removal of the second eye (Fig. 2). In the other 50%, removal of the first eye resulted in at most a transitory lengthening of τ while removal of the second eye produced a persistent lengthening (Fig. 3). We have not yet made a systematic study of this phenomenon, and in the experiments reported here have not kept track of whether the right or left eye was removed first. It therefore seems reasonable to assume that the right eye was removed first in about 50% of the experiments. While there are several possible explanations of this observation, perhaps the most intriguing is that there is an asymmetry in the neural input from the eyes to the circadian system. If there were right and/or left eyed birds in this sense, use might be made of this fact in tracing the neural pathways involved.

All of the information obtained in this study is consistent with the view that the effects of constant light on τ result from summation of input from the eyes with that from the ERP. The free-running period changes in the direction predicted by "Aschoff's Rule" when light intensity to the eyes is held constant and that to the ERP is varied or when light intensity to the ERP is held constant and that to the eyes is varied. This pattern of interaction between the two routes for photic input to the clock is similar to that which we have described for sparrows when entrainment rather than "Aschoff's Rule" is employed as an assay (McMillan, Keatts and Menaker, 1975) but differs from that found when either the photo-
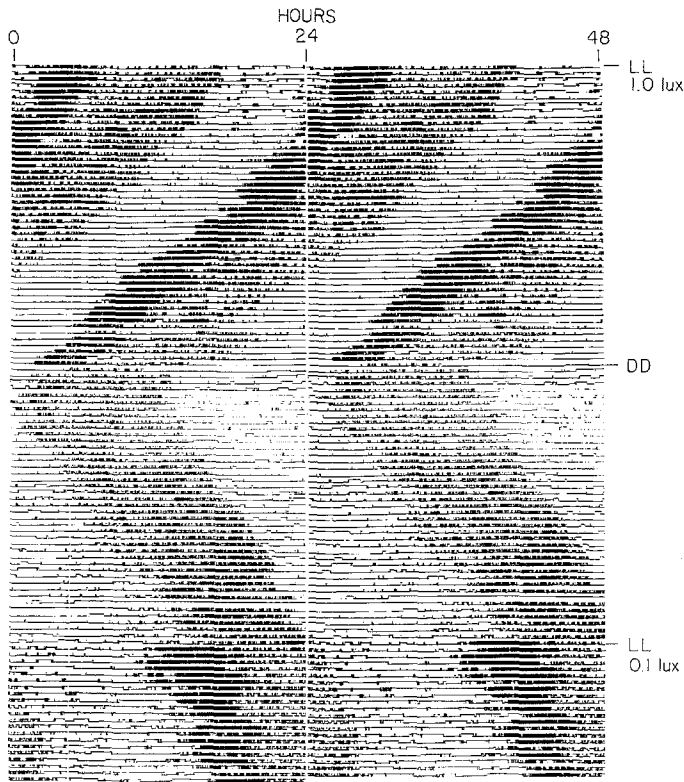
Fig. 1. "Aschoff's Rule" in an untreated House Sparrow. The perch hopping activity record has been duplicated to assist in following changes in the free-running period (τ) of the rhythm. Tau is less than 24 hours in LL (1.0 lux), greater than 24 hours in DD (constant darkness), and near 24 hours in LL (0.1 lux)

Table 1. The participation of both the eyes and the extraretinal brain photoreceptors of the House Sparrow in "Aschoff's Rule"

Initial conditions of birds (total)	Constant light[a] intensity (lux)	Treatment	Effect on period[b]
Normal (10)	DD	None	>24 hrs
Normal (5)	F (0.1)	None	~24 hrs
Normal (7)	F (1.0)	None	<24 hrs
Normal (12)	I (1.0)	Blinded	Lengthen
Normal (4)	F (1.0)	Blinded	Lengthen
Blind (5)	I (1.0, 10.0)	Head Hooded	Lengthen
Blind (4)	F (1.0)	Head Hooded	Lengthen
Blind (12)	I (1.0, 10.0, 2000.0)	Head Plucked	Shorten
Blind-Hooded (4)	I (1.0, 10.0)	Hood Removed	Shorten
Blind-Hooded (1)	F (1.0)	Hood Removed	Shorten
Normal (8)	P (0.1)	Head Plucked	Shorten
Normal (8)	P (0.1)	Carbon Black Injected	Lengthen
Normal (3)	F (1.0)	Saline Injected	None

[a] DD = constant darkness, F = fluorescent, I = incandescent, P = panelescent.
[b] The effects of the various treatments on the free-running period (τ) described here ("Lengthen", "Shorten", or "none") were found in *all* birds within each group.
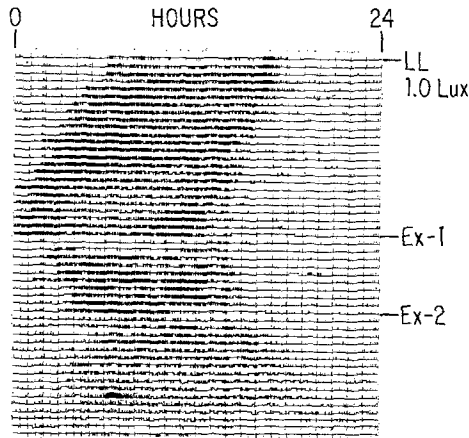
Fig. 2. "Aschoff's Rule" and the effect of blinding on the free-running activity rhythm of a sparrow in constant dim light (LL, 1.0 lux). The period of the rhythm (τ) is less than 24 hours prior to blinding. τ becomes longer than 24 hours after the bird is blinded. (Ex-1, removal of first eye; Ex-2, removal of second eye)
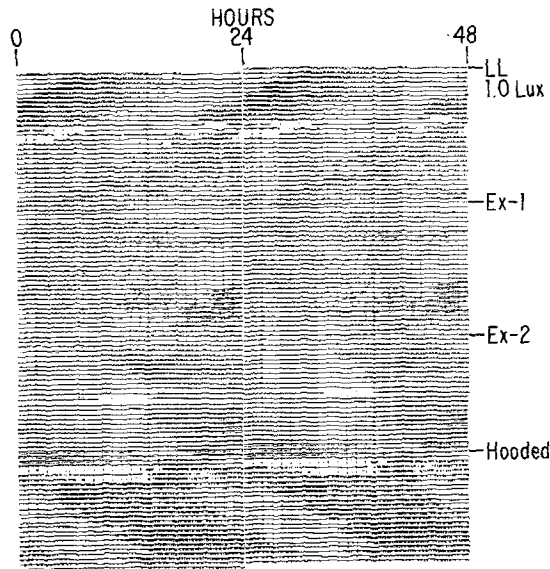

Fig. 3. The sparrow activity record has been duplicated to assist in following changes in the free-running period (τ) of the rhythm. Under LL of 1.0 lux the rhythm free-runs with a short period ($\tau < 24$ hours). After removal of the eyes τ lengthens ($\tau > 24$ hours). Note that in this record, in contrast to Fig. 2, significant lengthening of τ does not occur until after removal of the second eye (see text). Hooding of the head reduces the level of brain illumination producing complex transients and further lengthening τ

periodic response of the testes (Menaker *et al.*, 1970; McMillan *et al.*, 1975) or the production of arrhythmic locomotor activity by bright constant light is measured (McMillan, Elliott and Menaker, 1975). Underwood, in his studies of the effects
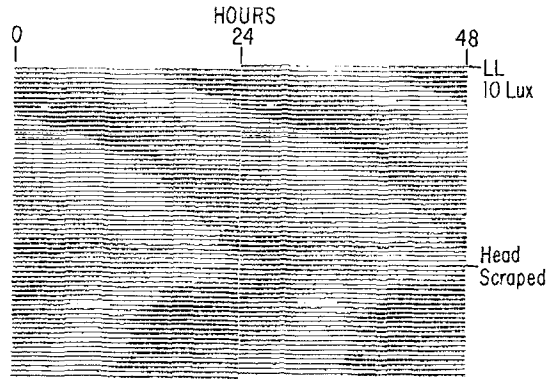
Fig. 4. The free-running rhythm of a blind-hooded bird exposed to LL (10.0 lux). The period of the rhythm is quite long ($\tau > 24$ hours) until the hood is removed when it shortens dramatically ($\tau < 24$ hours). Hood removal also produces an advancing phase shift. Similar shortening of τ was obtained by plucking the head feathers of blind birds (see Fig. 2 in McMillan, Elliott and Menaker, 1975)
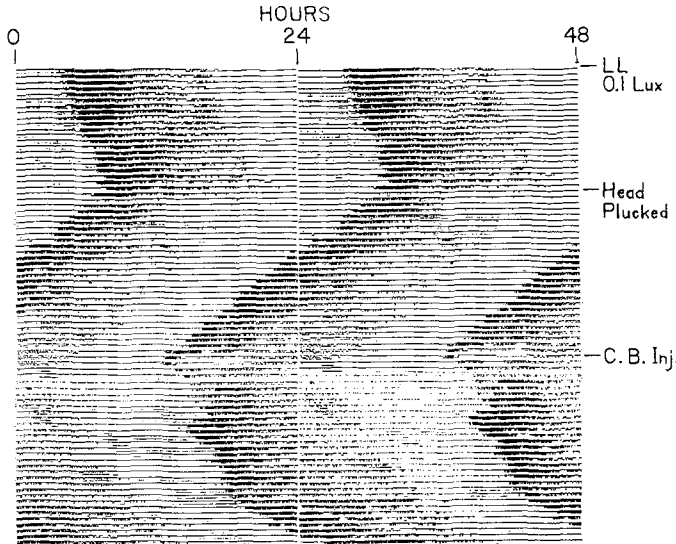

Fig. 5. The free-running period of a sighted sparrow (eyes intact). Under continuous illumination (LL, 0.1 lux "panelescent") τ is slightly longer than 24 hours. Head feather plucking shortens the period ($\tau < 24$ hours). Carbon black injection under the skin of the skull reverses the effect of plucking, lengthening the period ($\tau > 24$ hours)

of constant light on the free-running periods of the circadian rhythms of two species of lizards, has found two distinct patterns of interaction between photic input through the eyes and the ERP, each of which differs from that reported here for sparrows (Underwood and Menaker, in press). It is by now clear that the relative contributions to the perception of the photic environment made by the

eyes and the brain photoreceptors is highly variable among species and varies widely even in the same species if the regulation of different physiological processes is considered.

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