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On the Role of Eyes and Brain Photoreceptors in the Sparrow: Entrainment to Light Cycles

Joseph P. McMillan*, Henry C. Keatts**, and Michael Menaker

Department of Zoology, University of Texas, Austin, Texas, USA

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Summary. The eyes and extraretinal brain photoreceptor(s) of the House Sparrow (*Passer domesticus*) both contribute to entrainment of the locomotor rhythm. During exposure to a light cycle of low intensity 25 sparrows were entrained and eight free-ran (Table 1). Birds free-running under these conditions became entrained after the intensity of light reaching the brain was increased by plucking their head feathers (Fig. 1). Following carbon black injection beneath the skin over the skull of 23 of these entrained birds, 13 remained entrained and 10 free-ran (Figs. 1 and 2). The injected-entrained birds resynchronized to a 6 hour phase delay in the light cycle. Six of these birds were blinded (bilateral orbital enucleation) and subsequently free-ran. When the carbon black was then removed they re-entrained (Fig. 3).

Introduction

Light profoundly influences the reproductive physiology and the circadian clocks of many vertebrates, including the House Sparrow, *Passer domesticus* (see Menaker, 1971). Seasonal changes in daylength drive the annual reproductive cycle. Circadian rhythms of locomotor activity and body temperature can be entrained (synchronized) to a light-dark (LD) cycle. Under constant illumination the period of the free-running rhythm obeys ''Aschoff's Rule'', shortening when the level of illumination is increased and lengthening when it is decreased. Exposure to constant bright light causes a high level of continuous, arrhythmic activity.

In addition to the retinal photoreceptors of the lateral eyes, the House Sparrow possesses one or more extraretinal brain photoreceptors which participate in the perception of the photic environment. An obvious and significant question is: What roles do extraretinal brain photoreceptors (ERP) play in mediating the effects of light? Some basic features of ERP function have been demonstrated. Testicular growth in House Sparrows exposed to long days is controlled exclusively by the ERP; the eyes are not involved (Menaker *et al.*, 1970; McMillan *et al.*, 1975). The activity rhythms of sparrows blinded by bilateral orbital enucleation can be entrained to light cycles and obey "Aschoff's Rule" but do not

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* Present address: College of the Virgin Islands, St. Thomas, U.S. Virgin Islands 00801, USA.

** Present address: Suffolk County Community College, Biology Department, 533 College Road, Selden, L.I., New York 11784, USA.

become arrhythmic in constant bright light (Menaker, 1968a). Thus the circadian clock derives photic information from two photosensory inputs, the eyes and the ERP.

Many aspects of the relationship between the two loci of light reception and the clock remain unexplored. Recent findings on the role of the eyes and the ERP in mediating "Aschoff's Rule" and arrhythmicity are presented elsewhere (McMillan, Elliott and Menaker, 1975a, b). The relative contributions of the eyes and the ERP to the entrainment of sparrow locomotor rhythms by light cycles are discussed here.

Materials and Methods

House Sparrows of both sexes were collected in the vicinity of Austin, Texas and caged in individual boxes for exposure to light cycles (see Menaker, 1965, for details). Perch-hopping activity was measured with an Esterline Angus event recorder. Each box was illuminated by an electro-luminescent panel (Sylvania "Panelescent Nite-Lite") partially masked with black plastic tape to provide approximately 0.03 lux of green light at perch height. A clock produced *LD 12*:12 or *LD* 6:18 light cycles. The temperature within the boxes was stable (23 °±2 °C) and continuous random noise (92 dB) was present in the room.

Light levels reaching the brain were increased by plucking feathers from the heads of unanesthetized birds. Light levels were decreased by injecting carbon black suspended in distilled water (0.5 to 1.0 ml) between the skin and skull. This treatment could be later reversed by cutting the skin on top of the skull and scraping away the carbon black deposit. Birds were blinded by a procedure similar to that described in Menaker (1968a) except that both eyes were removed in one operation.

Results

The results are summarized in Table 1. Of 33 birds exposed to green light cycles of approximately 0.03 lux in intensity, 25 entrained and eight free-ran. Birds (numbers 1, 4 and 5 in Table 1) which free-ran under these conditions, entrained after their head feathers were plucked (Fig. 1). Carbon black injection of 23 entrained birds caused 10 to free-run through the light cycle (Figs. 1 and 2). Entrainment resumed when the carbon deposit was removed (Fig. 1). The 13 injected birds which remained entrained, resynchronized to a 6 hour phase delay in the light cycle (Fig. 3). Six of these were blinded and subsequently free-ran until the carbon black was removed, whereupon they re-entrained (Fig. 3).

Discussion

The fact that locomotor rhythms of blinded sparrows can be entrained by relatively dim light cycles clearly demonstrates the existence of extraretinal photoreceptors coupled to the circadian clock (Menaker, 1968a, b). This early work also establishes that the photoreceptors are in the brain although more precise localization has not yet been achieved. Several pieces of evidence then available suggested, but did not prove, that the eyes were also involved in the entrainment response. While all normal birds entrained to LD 12:12 (0.1 lux green light), 50% of the enucleated birds free-ran under these conditions. Blind birds also differed from normal sparrows in showing negative phase angles to

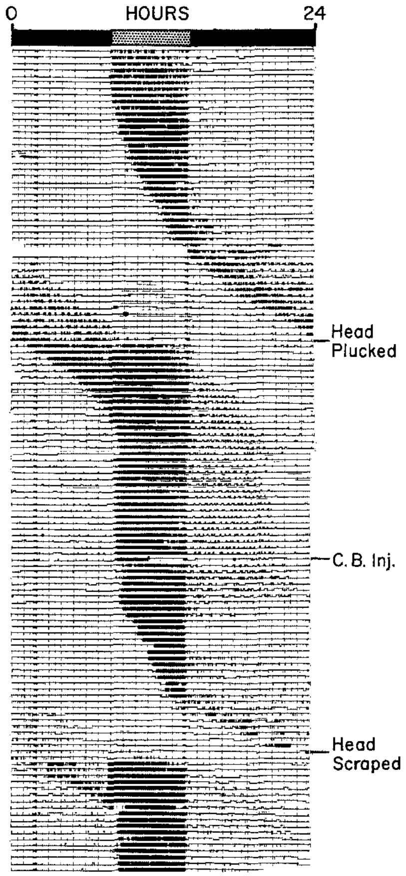
Table 1. The role of the eyes and the brain photoreceptor(s) in the entrainment to light cycles of the House Sparrow activity rhythm. By keeping track of bird numbers the responses of individual birds to sequentially applied treatments can be followed

No treatment: Bird Nos.	(total)	LD[a]	Response to light cycles
1–3	(3)	12:12	free-run
9–17	(9)	12:12	entrainment
4–8	(5)	6:18	free-run
18–33	(16)	6:18	entrainment
First treatment:			
Head feather plucking		Before treatment	Response to treatment
1, 4, 5	(3)	free-run	entrainment
Second treatment:			
Carbon black injection		Before treatment	Response to treatment
9–11	(3)	entrainment	free-run
18–24	(7)	entrainment	free-run
12–17	(6)	entrainment	entrainment
25–31	(7)	entrainment	entrainment
Third treatment:			
Light cycle phase shift		Before treatment	Response to treatment
12–17	(6)	entrainment	entrainment[b]
25–31	(7)	entrainment	entrainment[b]
Fourth treatment:			
Blinding		Before treatment	Response to treatment
15, 17	(2)	entrainment	free-run
28–31	(4)	entrainment	free-run
Fifth treatment:			
Carbon black scraping		Before treatment	Response to treatment
15, 17	(2)	free-run	entrainment
28–31	(4)	free-run	entrainment

[a] 0.03 lux green light, birds were held on these light cycles throughout the experiment.
[b] The LD cycle was delayed 6 hours and the birds re-entrained to it, see Fig. 3.

long photoperiods and in displaying a greater number of transient cycles before attaining an entrained steady state. Menaker explained these results by assuming that the removal of the eyes decreased the effective intensity of the light input to the circadian clock. In the present study we have examined these suggestions further. The work reported here is directed at testing three hypotheses: I. The ERP plays a role in the entrainment of intact as well as of blinded birds, II. The eyes play a role in entrainment, and III. Light evoked signals from the eyes and those from the ERP are summed to produce entrainment.

The free-running behavior shown by 25% of the normal birds exposed to low intensity light cycles indicates that 0.03 lux of green light is near the threshold for entrainment of intact birds. Plucking of the head feathers, which increases



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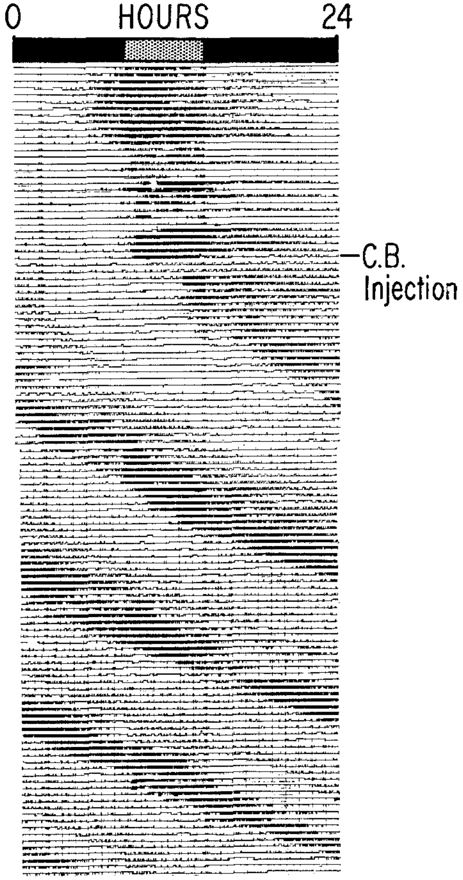

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Fig. 1. Perch-hopping record of a House Sparrow exposed to an LD 6:18 cycle. The cycle is diagrammed at the top of the record: black indicates darkness; stipling indicates green light of approximately 0.03 lux. The bird's activity rhythm escapes entrainment by the light cycle and free-runs until the head feathers are plucked. When carbon black is injected beneath the skin of the head the rhythm again free-runs. Entrainment is re-established after removal of the carbon black

Fig. 2. The activity rhythm of a sparrow which is entrained to an LD 6:18 cycle diagrammed as in Fig. 1. When carbon black is deposited beneath the skin of the head the rhythm free-runs, traversing the light cycle three times

the intensity of light reaching the brain by a factor of 10 to 100, led to entrainment of these birds via an augmented stimulation of the ERP. Of those intact birds which entrained to the 0.03 lux cycles, nearly half free-ran after injection with carbon black which reduces by 10 to 100-fold the light reaching the brain. When the carbon deposit was removed they re-entrained. Thus the entrainment response of sparrows with their eyes can be manipulated by changing, in either direction, the amount of light reaching the ERP. These results strongly support hypothesis I.

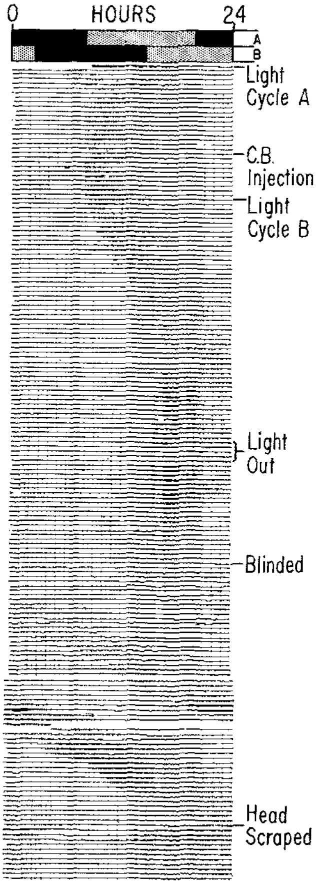

Fig. 3. The activity record of a sparrow exposed to the light regimens (LD 12:12, approximately 0.03 lux, green light) diagrammed at the top of the figure. Entrainment to light cycle A continues after carbon black is injected under the skin of the head. Re-establishment of the entrained steady state following the 6 hour phase delay (light cycle B) requires 36 days of transients. After blinding the activity rhythm free-runs through the light cycle twice. When the carbon black is removed the blind bird re-entrains to the light cycle

Carbon black injection considerably attenuates illumination of the ERP. The fact that 13 of 23 injected birds entrained to light cycles and subsequently re-entrained to a 6 hour phase shift, suggests that their entrainment was primarily mediated by retinally perceived light. This suggestion is confirmed by the fact that after blinding, which of course eliminates input to the clock from the eyes, these birds free-ran in the presence of the light cycle to which they had previously entrained. These experiments clearly support hypothesis II.

Although our information concerning the minimum light intensity necessary to support entrainment of sparrows which have been treated in various ways is crude, it nonetheless fits neatly into a reasonable pattern. Of 33 intact birds (neither blinded nor injected), 25 entrained to 0.03 lux light cycles (76%). Of

23 injected birds (in which the eyes provide the primary input to the clock), 13 entrained to 0.03 lux light cycles (57%). From previous work (Menaker, 1968a) it is clear that less than 50% of blinded birds (in which the ERP provides the only input to the clock) would have entrained to 0.03 lux light cycles. Taken together the facts 1) that intact birds are more sensitive to entraining signals than either blinded or injected birds and 2) that both the eyes and the ERP have been shown to contribute to entrainment in intact birds, support hypothesis III.

It is worth noting that the pattern of interaction between the eyes and the ERP which we have here demonstrated to operate in the control of entrainment, does not operate in the control of the photoperiodic response of the gonads of the sparrow (Menaker *et al.*, 1970; McMillan *et al.*, 1975) or, in fact, in the control of at least one other aspect of the response of the sparrows' clock to photic input (McMillan *et al.*, 1975b).

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Joseph P. McMillan
College of the Virgin Islands
St. Thomas, U.S. Virgin Islands 00801, USA

Henry C. Keatts
Suffolk County Community College
Biology Department
533 College Road
Selden, L.I., New York 11784, USA

Michael Menaker
Department of Zoology
University of Texas
Austin, Texas 78712, USA