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Extraretinal Light Perception in the Sparrow, III: The Eyes Do Not Participate in Photoperiodic Photoreception*

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Abstract. Photoperiodic control of testis growth in *Passer domesticus* (house sparrow) is mediated entirely by extraretinal photoreceptors in the brain. The eyes do not participate in photoperiodically significant photoreception. Removal of the pineal organ does not affect either the response to light or, to a first approximation, the process of recrudescence. The intensity of light reaching the retina and that reaching the extraretinal photoreceptor were varied independently. This technique will make it possible to study brain photoreception in species of birds that will not tolerate blinding. Extreme caution is necessary in the interpretation of brain lesion experiments in which reproductive function is modified, since photoreception by brain receptors of unknown anatomical location affects testicular state.

In an earlier paper in this series[1] we reported that seemingly normal testis growth occurred in *Passer domesticus* exposed to inductive daylengths even though the eyes had been surgically removed. We concluded that an extraretinal photoreceptor (ERR_p—extraretinal receptor for photoperiodism) must be involved in the control of testicular recrudescence and speculated on the role of *retinal* light perception in the intact bird. Benoit has argued that both a retinal and a brain photoreceptor are involved in the testis response of ducks.[2] We discussed his arguments and concluded that the question of whether the eyes were involved at all, remained open. We have since demonstrated[3] that there are no significant differences in either rate or extent of testis growth in blinded, as opposed to unoperated, sparrows held on the same lighting regimen. This result was confirmed at several different photoperiods, light intensities, and times of year; and strongly suggests, although it does not prove, that retinal light perception is not involved in photoperiodically-mediated reproductive control in *P. domesticus*.

We have shown that the synchronization (entrainment) of the circadian rhythm of activity in the sparrow is also mediated by an extraretinal light receptor (ERR_e—extraretinal receptor for entrainment).[4] Further, this receptor must be in the brain, as the behavioral response to light cycles can be manipulated by affecting the amount of light that penetrates the head.[5]

The present paper applies techniques that have been shown to affect the amount of light reaching the ERR_e to the study of photoperiodically-controlled

testis growth. The experimental design was based on two assumptions: 1) that the ERR_p, like the ERR_e, is located in the brain, and 2) that the eyes play no part in the perception of light cycles as those cycles affect reproductive state. Both assumptions are clearly confirmed by the experimental results. In addition to demonstrating that retinal light perception is not involved in this aspect of the reproductive cycle of the house sparrow, the techniques reported below make it possible to study extraretinal light perception in any species of bird whether or not it will tolerate blinding. These techniques should therefore remove a major obstacle to further progress in our understanding of this facet of the functioning of the avian brain.

Results. In previous work with the circadian activity rhythm of sparrows we have shown that plucking feathers from the top of the head increases the intensity of light reaching the ERR_e, and injection of india ink under the skin of the head decreases that intensity.[5] In the present study we combined both these techniques with the use of a stimulatory photoperiod at a light intensity very close to the threshold for photoperiodic response in normal sparrows.[6] We exposed two groups of sighted birds to the same ambient light intensity, but completely occluded the ERR_p's of one group while exposing the ERR_p's of the other. We obtained no testis growth in the first group and significant growth in the second despite the fact that retinal perception in the two groups was identical.

The birds in experiment I were divided into two groups. All feathers were plucked from the tops of the heads of one group while the other group received injections of india ink under the skin of the head.[7] Neither group was blinded and both groups were maintained, for the duration of the experiment, in the same room on *LD 16*:8. The intensity during the light portion of the cycle varied from 7–14 lux depending on the position of the particular cage in the room. The birds were so distributed in the room that each group received approximately the same average light intensity. The experimental treatment began on January 3, 1969: the experiment was terminated and the birds killed 39 days later.

The mean testis weights of both experimental groups, as well as the mean testis weight of a control group drawn from the same population of birds and sacrificed at the beginning of the experiment (initial controls), are shown in Table 1. Note that extensive testis growth occurred in the group of birds whose head feathers had been plucked whereas no growth occurred in the injected group.

Experiment II was designed to assess the possible role of the pineal organ as an ERR_p. A group of initial controls and four experimental groups of birds were

TABLE 1. *Effects of manipulating the intensity of light reaching the ERR_p on the testis weights of sparrows with intact eyes.*

	Initial controls	India ink injections	Head feathers plucked
Testis weight	6 ± 1.5 (N = 11)	8 ± 2.3 (N = 27)	323 ± 24 (N = 28)

* Birds were held on *LD 16*:8, at an approximate intensity of 10 lux during the light phase, for 39 days. Testis weights are means (combined weights of both testes in mg) of all birds in the group, followed by the S.E. and the number of birds in the group. Initial controls were sacrificed at the start of the experiment.

used: pinealectomized plucked (PP), pinealectomized injected (PI), sham-operated plucked (SP), and sham-operated injected (SI).[8] None of the birds were blinded. The results of this experiment are shown in Table 2 and are discussed below.

TABLE 2. *Effects of pinealectomy and sham pinealectomy on testicular recrudescence mediated by the ERR_p.*

	Testis weight*	Range
Pinealectomized plucked (PP)	281 ± 14 (N = 16)	150–383
Sham-operated plucked (SP)	283 ± 29 (N = 5)	195–372
Pinealectomized injected (PI)	130 ± 26 (N = 15)	3–358
Sham-operated injected (SI)	179 ± 71 (N = 5)	1–384
Initial controls	4 ± 1 (N = 11)	2–7

* Testis weight values are given as in Table 1. The highest and lowest value for each group is given under "Range." See footnote 8 for experimental details. Differences are significant between groups PP and PI ($p < 0.05$—an approximate t test was used because the variance differed significantly), SP and PI ($p < 0.001$), and combined PP, SP vs. combined PI, SI ($p < 0.001$—approximate t). Statistical procedures are from *Principles and Procedures of Statistics* by R. G. D. Steel and J. H. Torrie (New York: McGraw-Hill, 1960).

Locomotor activity data were collected from some of the birds in the first experiment and all of the birds in the second. In all cases the rhythm of locomotor activity was entrained by the light cycle and was phased approximately normally to it.

Discussion and Conclusions. Experiment I demonstrates that light perception by the retina is not involved in photoperiodic control of testicular recrudescence in the house sparrow. Both groups of birds were exposed to artificial long days at the same average light intensity. Despite this retinal exposure to light, the testes of the group whose brains were shielded from exposure to light by means of india ink injections failed to respond. The testes of the group with feathers plucked from the heads grew dramatically. The shielding effect of india ink injections and the decrease in opacity caused by plucking feathers have both been verified by direct physical measurement. As each bird was sacrificed at the end of the experiment, the brain was aspirated from the braincase and light that penetrated the dorsal surface of the head (feathers, skin, ink, and skull in the injected group, and skin and skull only in the plucked group) was measured with a photomultiplier.[9] These measurements indicate that ink injections *reduced* the intensity of light that penetrated the dorsal surface of the head to about a tenth of the intensity that penetrated the head of untreated birds. When feathers were plucked, light penetrance was *increased* from 100 to 1000 times above its penetrance through the heads of untreated birds. In experiment I, therefore, the plucked birds received illumination to the ERR_p of about 1000–10,000 times greater intensity than that to the ERR_p of the injected birds.

The ERR_p, which alone mediates the sparrow's response to photoperiodic stimuli, must be located in the brain. Birds whose brains are shielded from light by ink injections fail to respond. We had earlier reached the same conclusion (on the basis of technically similar experiments) with regard to the ERR_e.[5] That these two brain receptors may not be identical is suggested by the facts

1) that threshold intensities required to elicit the two responses differ in the untreated bird by approximately 100 times (less than 0.1 lux for entrainment and ~10 lux for the photoperiodic response), and 2) that the eyes appear to be involved in entrainment[4] but are not involved in photoperiodic photoreception.

In the house sparrow, the route by which light normally reaches the ERR_p must lie preferentially through the dorsal surface of the head. When this avenue is closed, not enough light penetrates through other sites to cause a response, at least at the low intensities used in our experiments. Of course, field intensities are so much higher than our experimental ones that it is not unreasonable to suppose that in the field light floods the brain through many routes.

Insofar as our results apply to other species of birds, they suggest that extreme caution is necessary in the interpretation of experiments in which brain lesions are used to study the possible role of various areas of the brain in the neuroendocrine control of testicular recrudescence. An extensive literature has grown up in this area, resting on the assumption that a lesion that interferes with testis growth does so by destroying neuroendocrine centers involved in the control of this process. If, however, only brain photoreceptors are involved in the perception of light stimuli, then all lesion experiments are open to the alternative, and equally reasonable interpretation, that what has been destroyed is in fact the ERR_p itself, or some portion of the pathway that links the ERR_p with appropriate neuroendocrine centers.

Unpublished experiments on *P. domesticus* from this laboratory,[10] as well as work from other laboratories on other species of passerine birds,[11] indicate that pinealectomy does not interfere with testicular recrudescence. Since the eyes are not involved in photoperiodic photoreception, these results suggest that the pineal gland is not the ERR_p (or at least not the only ERR_p). Experiment II was designed as a direct test of this hypothesis.

It is evident from Table 2 that pinealectomy did not affect recrudescence (compare testis weights of groups PP and SP and those of groups PI and SI). The photoperiodic response, which we know on the basis of experiment I is mediated entirely by brain photoreceptors, occurs whether or not the pineal is present; thus the pineal cannot be the sole ERR_p.[12] This strong inference appears to require only one qualification: it is possible that the pineal is photoreceptive in the intact bird but that its removal shifts the locus of photoreception. At present we cannot exclude this possibility as it applies to another brain photoreceptor, but we can exclude it as it applies to the retina. If pinealectomy shifted the locus of photoreception to the retina, then the testes of pinealectomized injected birds should show a greater response to the inductive light cycle than those of sham-operated injected birds. In fact, the two groups do not differ.

Since pinealectomy has no effect on recrudescence, we can combine the pinealectomized plucked with the sham-plucked groups and test them statistically against the combined pinealectomized-injected and sham-injected groups. The testis weights of these combined groups are significantly different ($p < 0.001$). This result confirms the result of experiment I; the difference is obviously due to differences in the amount of light perceived by the ERR_p and not the light

(identical in all birds) perceived by the eyes. However, the results of experiment II differ importantly from those of experiment I in that the testes of both injected groups grew significantly from the level at which they began the experiment (indicated by the initial controls). This is almost certainly due to unanticipated difficulties encountered in making effective ink injections on the heads of birds previously subjected to surgery. Although the heads of these birds seemed in good condition at the start of the experiment, by the time the experiment was terminated almost all the injected birds had lost most of their head feathers; gaps in the ink cover were often easily visible, and local infections were sometimes present in the skin of the head. None of these difficulties was encountered with the injected (but unoperated) birds in experiment I. The very wide range of testis weights in both the injected groups of Table 2 supports this interpretation, as the light leaks which may have occurred might well be located at random with respect to the unknown location of the ERR_p.

Oishi and Kato[13] have suggested that the pineal organ may be an ERR_p in Japanese Quail (*Coturnix coturnix japonica*) on the basis of experiments in which radioluminous paint applied to the skull over the pineal region maintained testis weight in unoperated birds, but failed to do so if the pineal had been removed. Their data are subject to the alternative interpretation that pinealectomy physically interferes with the penetrance of light to other areas of the brain in which the ERR_p might be located. Using a modification of their technique, Homma[14] has performed more precise experiments on Japanese Quail. He concludes that neither the retina nor the pineal organ is involved in photoperiodic photoreception. Homma's results are thus consistent with ours on both these important points.

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Abbreviations: ERR_p, extraretinal receptor for photoperiodism; ERR_e: extraretinal receptor for entrainment; *LD 16*:8, a light-dark cycle of 16 hr light and 8 hr darkness per 24 hr day.

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[1] Menaker, M., and H. Keatts, *Proc. Nat. Acad. Sci. USA*, **60**, 146 (1968).
[2] Benoit, J., *Ann. N. Y. Acad. Sci.*, **117**, 204 (1964).
[3] Underwood, H., and M. Menaker, *Science*, **167**, 298 (1970).
[4] Menaker, M., *Proc. Nat. Acad. Sci. USA*, **59**, 414 (1968).
[5] Menaker, M., *Proc. American Psychological Association, 76th* Annual Convention, 299 (1968).
[6] Bartholomew, G. A., Jr., *Bull. Mus. Comp. Zool. Harvard Univ.*, **101**, 433 (1949).
[7] Feathers were plucked at two week intervals during the course of the experiment. India ink injections were also given in the breast muscle of half of the birds in the plucked group as a control on possible direct effects of the ink on testis growth. These injections had no effect on testis growth.
[8] The birds were held on *LD 8*:16 for about one month prior to the beginning of the experimental photoperiod. During this period pinealectomies and sham operations were performed. The sham operation acted as a control for the effects of the surgery on the condition of the scalp and skull with regard to light penetration. Although a piece of the skull was removed as though for pinealectomy, the meninges and the brain were not disturbed. On January 6 1970, the birds were either plucked or injected and were placed in individual light-tight boxes on

LD 16:8, at an intensity of between 5 and 6 lux measured four inches above the floor of the cage. The experiment was terminated and the birds killed on February 16, 1970.

[9] Photomultiplier manufactured by Photo Research Corporation, Hollywood, Calif.

[10] Gaston, Suzanne, and M. Menaker, unpublished experiments (1967).

[11] Farner, Donald S., personal communication.

[12] The completeness of pinealectomy was confirmed histologically. Sections (8–12 μm) stained in Ehrlich's hematoxylin and eosin were examined by light microscopy. Pineal removal was complete in most cases. Although some organized pineal tissue could occasionally be found, the conclusion remains inescapable that apparently normal testis growth can occur in the absence of any identifiable pineal tissue.

[13] Oishi, T., and M. Kato, *Mem. Fac. Sci., Kyoto Univ., Ser. Biol.*, 2, 12 (1968).

[14] Homma, K., in *Biochronometry*, ed. M. Menaker (Washington, D.C., Academy of Sciences), in press.