EXTRARETINAL LIGHT PERCEPTION IN THE SPARROW, II. PHOTOPERIODIC STIMULATION OF TESTIS GROWTH*

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The response of avian reproductive organs to photoperiod both in the field and in the laboratory has been the subject of continuing investigation since its discovery by Rowan in 1926.[1] The great majority of avian species go through an annual reproductive cycle in which one of the most dramatic events is a rapid growth (recrudescence) of the male gonads in early spring preparatory to the breeding season. During this phase of the annual cycle the testes may increase in weight from their regressed winter condition by a factor of 500 or more. Although the precise manner by which day length influences the endocrine system remains to be worked out, it is abundantly clear that light plays a crucial role in stimulating recrudescence.

In a series of papers spanning more than 30 years, Benoit and his colleagues have reported on experiments which demonstrate that a photoreceptor other than the retina is involved in the photoperiodic response of the testis of the duck.[2] Benoit's data led him to conclude that the extraretinal photoreceptor is localized in the hypothalamic area of the brain and that both it and the retina normally participate in what he has called the optosexual reflex.

Although Benoit's work remains the only evidence for the participation of an extraretinal photoreceptor in the control of avian reproductive cycles, there are indications that such receptors may be present and act in a similar manner in other widely diverse groups of animals. Lisk and Kannwischer[3] have shown that the estrous cycle of the rat can be modified by light impinging directly on the hypothalamus. Lees[4] has demonstrated that modification of the development of aphids toward the sexual form occurs when light in an appropriate photoperiodic regimen is directed to the brain and concludes that the eye is not involved. Referring to the photoperiodic response of arthropods in general, Lees states: "There is no evidence that the light pathway ever involves the simple or compound eyes."

The discovery of an extraretinal photoreceptor which mediates the entrainment by light of the circadian rhythm of locomotor activity in the sparrow *Passer domesticus*[5] led us to investigate the possibility that such an extraretinal receptor might also be involved in the well-known response of the reproductive system of this species to photoperiod. This paper reports on experiments which demonstrate that stimulation of testicular recrudescence by "long days" occurs in blinded as well as in normal house sparrows.

Materials and Methods.—Male house sparrows were collected in and around Austin and held in outdoor aviaries until the beginning of experimental treatment. They were enucleated as previously described[5] and, during experiments, were maintained in individual cages in separate light-controlled boxes at 23° ± 2°C. Light sources were 4-watt "cool white" fluorescent bulbs (Ken Rad F4T5/cw) which produced an intensity of about 500 lux at perch level. Perching activity of all birds was recorded continuously during the experiments. At the end of the experiments, the birds were killed, their testes were removed, and the combined weight of the two testes from each bird was obtained. The testes were fixed in Bouin's, sectioned at 5 μ, and stained in Mayer's hemalum and eosin.

Results.—Bartholomew in 1949[6] established that testicular recrudescence is photoperiodically controlled in the house sparrow. Figure 1 shows this response for normal birds in our hands.

As a test of the hypothesis that an extraretinal receptor is involved in the control of the testicular response to photoperiod, an experiment was undertaken to compare the response of normal unoperated sparrows to that of bilaterally enucleated birds under short and long photoperiods. In order to maximize possible

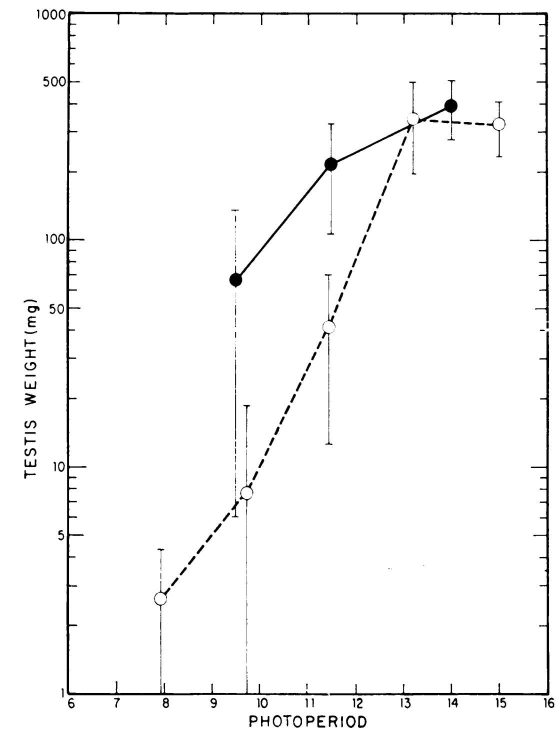

Fig. 1.—The testicular response of normal sparrows to photoperiod at two times of year. The dashed line describes the response in an experiment in which photoperiodic treatment was initiated on 11/4/66 and was continued for 46 days before the birds were sacrificed. The experiment described by the solid line began on 1/16/67 and was terminated 56 days later. In both experiments the birds were isolated in individual cages and light was provided as described in *Materials and Methods.* Points are means of the combined weights of the two testes of all birds in each group. Bars are standard deviations. (Menaker and Eskin, unpublished experiments.)

differences, the photoperiods chosen were LD *6*:18 and LD *16*:8. As a control for the effects of enucleation, a group of blind birds was held in constant darkness for the duration of the experiment. Twelve birds chosen at random from the outside aviaries were assayed at the beginning of the experiment as a check on the initial state of testis development of the experimental population. During the first two weeks of December, one eye was surgically removed from those birds to be bilaterally enucleated, and they were returned to an outdoor aviary. On December 29, all the birds were placed in the experimental boxes on LD *9.5*:14.5 (sunrise in Austin on December 29 occurred at 7:27 A.M. and sunset at 5:39 P.M.). On January 1, the second eye was removed, and the experimental photoperiods began on January 2. Sixty-one days later the experiment was terminated and the birds were killed. Table 1 shows the results of this experiment. A comparison of the testis weights of blind birds in constant darkness or on 6 hours of light per day with the testis weights of blind birds on 16 hours of light per day makes it immediately apparent that the testes of blind birds responded to the long photoperiod. Further, when the blind birds on both short and long photoperiods are compared with the normal unoperated birds, it is clear that, under these experimental conditions, there are no significant differences between the testicular responses of birds with and without eyes. Unfortunately, three of the five normal birds which began the experiment on LD *16*:8 died during its course, making quantitative comparisons at this photoperiod statistically unreliable. However, the average testis weight of the blind birds on LD *16*:8 is very close to that which one would expect for normal birds on the basis of the data plotted in Figure 1. It is clear from the above that the testicular response of bilaterally enucleated birds to LD *16*:8 is, if not identical with, at the least very similar to that of birds with eyes.

Histological examination revealed no obvious abnormalities in the testes of blind birds on either photoperiodic regimen (Fig. 2). Mature sperm were present in large numbers in the testes of all birds, blind and sighted, exposed to LD *16*:8. No histological differences were found among the three other groups of birds in the experiment. Most testes were in stage 2,[6] with an occasional stage 1 or 3 appearing in each group. Some slight testis development on short photoperiods and in constant darkness is to be expected in *P. domesticus*, especially toward the end of winter.[7, 8]

All birds were weighed at the conclusion of the experiment, and while there is a tendency for the blind birds taken as a single group to be somewhat lighter than the normal birds, no weight differences were found between groups on the different photoperiods. Also, small differences in the phase of the daily activity onset of the perching rhythm were found between the blind and sighted birds on both long and short photoperiods.[5]

Discussion.—The unstated assumption that visible light is the causal agent of photoperiodic induction by artificial light cycles is universal among workers in avian photoperiodism. We see no reason to question it at present but feel that it should be made explicit. On this assumption our results demonstrate clearly that *P. domesticus* possesses an extraretinal photoreceptor(s) (ERR) which is coupled to the neuroendocrine system controlling the annual reproductive cycle.

Recent work has made it clear that there is a relationship between the "clock" controlling circadian rhythms and the timing involved in the measurement of day

TABLE 1. *Effects of several light regimens on testis weight of blinded and normal sparrows.**

	Constant darkness	LD *6*:18	LD *16*:8
Bilaterally enucleated	17 ± 20 (6)	15 ± 12 (9)	404 ± 106 (12)
Unoperated		10 ± 4 (5)	426 (2)
Initial controls		7 ± 4 (12)	

* The numbers in each category are the mean testis weight (both testes) in mg. of all birds in the group, followed by the S.D. and, in parentheses, the number of birds in the group.

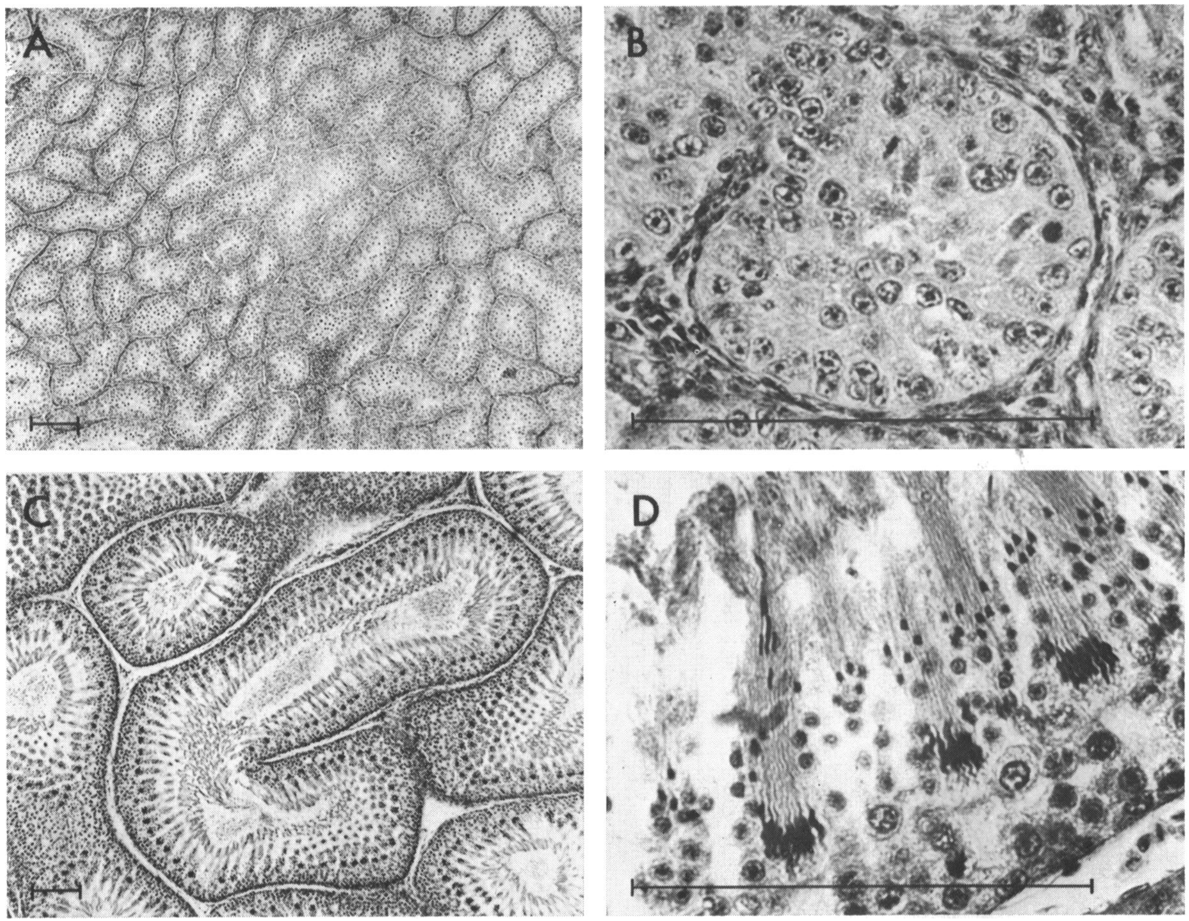

FIG. 2.—Histological sections from the testes of blinded birds exposed to LD *6*:18 (*A* and *B*) and LD *16*:8 (*C* and *D*).

(*B*) and (*D*) have been photographed at approximately 10 times the magnification used in (*A*) and (*C*). Bars indicate 10 μ in all cases.

Note the tremendously hypertrophied tubules in (*C*) as compared with (*A*), as well as the mature sperm present in (*D*).

length as assayed by the testis response in *P. domesticus*.[9] Whether the ERR responsible for the entrainment by light cycles of the circadian rhythms of locomotor activity of blinded sparrows is identical with the ERR which mediates their photoperiodic response remains an open question.

Our work is the first confirmation with a different species of the long-standing conclusion of Benoit and his colleagues that an extraretinal receptor participates in the "optosexual reflex" of the duck. The discovery of such a receptor in a passerine bird significantly increases the probability that birds in general possess and use them.

Benoit's conclusion[2] that both the eyes and an extraretinal receptor participate in the optosexual reflex must for the present be viewed with caution. Benoit deduces a retinal contribution from a comparison of the responses of birds with opaque masking materials inserted behind the eye and in which the optic nerves are either sectioned or left intact. The route by which light reaches the ERR in unoperated birds remains unknown. If, as seems possible, the eye functions not as a receptor, but as a major "window" to the ERR, Benoit's interpretation of these experiments is open to question. The greater response which Benoit finds in birds with intact optic nerves may be due either to the relative difficulty of masking the orbit in birds with intact nerves or to light—as light rather than as nerve impulses—traveling along the nerve itself. Sectioning the nerve may induce degenerative changes which could reduce the intensity of the light reaching the ERR to below its value in normal birds. Our data throw no further light on this problem despite the fact that the magnitude of the response which we obtained appears to be independent of the presence of the eyes. In the interpretation of our results, the distinct possibility remains that the testes of both the blind and the normal birds on LD *16*:8 have different growth curves and we have simply assayed them at a point in time where the two curves have leveled off together. However, in the light of present inadequate knowledge, it would appear reasonable to suspend the generally held assumption of retinal involvement in avian photoperiodism until convincing evidence is available.

The question arises as to whether the statement of Lees, quoted above, can be extended to include all animal photoperiodic systems. Extraretinal photoreception has been shown to participate in reproductive responses to light in arthropods, birds, and mammals. Is there in fact convincing evidence that the eye participates in any such response?

In mammals it seems clear that retinally perceived light does exert at least partial control of the reproductive state.[10] One case in which the anatomical connections between the retina and the neuroendocrine centers have been carefully investigated holds great interest for the present discussion.

In rats, constant light suppresses the melatonin synthesizing activity of pineal hydroxyindole-O-methyltransferase (HIOMT) relative to its activity in constant darkness. Melatonin in turn inhibits gonadal function. Constant light retinally mediated therefore appears to stimulate reproductive function by inhibiting the formation of melatonin.[11]

Moore, Heller, Wurtman, and Axelrod[12] have shown that the effect of constant light on melatonin synthesis is mediated solely by the inferior accessory optic tract which "separates from the primary tract just beyond the optic chiasma and enters the lateral hypothalamus to run caudally among the fibers of the medial forebrain bundle before terminating in the rostral midbrain tegmentum." Rats in which only the primary optic tracts were destroyed *behaved* in response to visual stimuli like blinded animals but retained the normal HIOMT response to light; rats in which only the inferior accessory tracts were sectioned *behaved* like normal sighted animals but, as in bilaterally enucleated animals, constant light failed to suppress their HIOMT activity.

Functional ERR have been retained by arthropods, birds, and mammals, despite the presence of highly organized photoreceptors. In the case of the rat, in which the contribution of the retina has been well documented, its contribution to reproductive control is anatomically and physiologically separate from its image forming capacities. One is led to speculate that there may be as-yet unappreciated selective advantages in processing information, derived from the environmental light cycle and used in the regulation of reproduction, independently of other visual information of more immediate temporal importance.

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