SHORT COMMUNICATIONS

INEFFECTIVENESS OF PINEAL LESIONS ON THE TESTIS CYCLE OF A FINCH

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In recent years the pineal gland has become increasingly associated with aspects of reproductive physiology (Wurtman et al. 1963; Chu et al. 1964; Ebels and Prop 1965; Hoffman and Reiter 1965a, b; Reiter and Hester 1966; Reiter et al. 1966; Reiter 1967a, b; McFarland et al. 1968a). Of these investigators, Wurtman and Axelrod (1965) appear to have formulated the most general hypothesis regarding pineal control. They suggested that control of the reproductive cycle is mediated by the pineal gland. According to their scheme, light inhibits synthesis of melatonin in the pineal gland which, in turn, inhibits gonadal growth. Thus, an increase in light (or day length) would cause a decrease in melatonin synthesis, and this decrease in inhibitory melatonin would result in reproductive maturation. A lack of inhibitory light (short days) presumably induces testicular or ovarian atrophy by permitting an increase in inhibitory melatonin. Both melatonin synthesis (Fiske 1964; Quay 1964; Snyder et al. 1964a, b; Snyder and Axelrod 1965; Reiter et al. 1966) and the photoperiodic response of birds (Hamner 1963, 1964, 1966; Farner 1965; Menaker 1965; Wolfson 1965) appear to be moderated by circadian rhythms and, as suggested by Wurtman and Axelrod, the circadian melatonin rhythm may act as the biological clock for seasonal breeding.

Whereas most of the available evidence supporting the above hypothesis came from studies of rats and hamsters, recent investigations on Japanese Quail (*Coturnix coturnix japonica*) suggest that reproduction in birds may also be affected by the pineal gland (Shellabarger 1952, 1953; Homma et al. 1967; McFarland et al. 1968a, b). To our knowledge, however, there are no published experiments that attempt to disprove for birds the following specific null hypothesis: the pineal gland has no effect on photoperiodically induced testicular growth, regression, or termination of photorefractoriness in a non-domesticated, normally cyclic bird.

We are privately aware of at least several unsuccessful attempts to disprove aspects of this hypothesis, but these data have not been published. Since much attention is given to the possibility of pineal control of avian testis cycles, we are prompted to present our own negative evidence. In two experiments we found that severe pineal lesions were without any obvious effect on photoperiodically induced testicular growth or on termination of photorefractoriness in the House Finch (*Carpodacus mexicanus*).

METHODS AND MATERIALS

Male House Finches in one of two reproductive states were used in the two experiments; birds were maintained and handled with techniques and equipment described previously (Hamner 1966). Experiment I, concerned with testicular growth, began on 25 June. These birds had been held on an LD 6:18 lighting regimen since their capture the previous fall. One group was exposed to LD 6:18, the second to LD 12:12. After 28 days all were killed and their testes weighed. Experiment II examined termination of photorefractoriness, and used freshly captured birds that were maintained on a constant-light regimen until 11 August. Thereafter some of the birds were placed on an LD 6:18 regimen and others on LD 18:6. After 65 days all birds were exposed to LD 18:6 for 21 days then killed, and the left testis weighed. Control birds received lesions in the right or left cerebral hemisphere, and experimentals, directly in the pineal gland from a D.C. lesion maker at 3 ma for 30 sec. Extent of pineal lesions was assessed by histological section of brains of experimental birds or by gross dissection.

RESULTS AND DISCUSSION

Experiment I was designed to test the possible effect of pineal lesions on testicular growth. According to Wurtman and Axelrod's hypothesis, if the pineal was removed or severely damaged, less inhibitory melatonin would be produced and testicular growth would occur. We would expect, therefore, that the testes of groups of birds with pineal lesions would immediately enlarge whether on long days or short days. Similar testicular responses of the controls and experimentals in long and short day groups, would be inconsistent with their hypothesis. Six birds, laparotomized before the experiment began, had a left testis with a mean length of 1.0 mm. Table 1 presents the results. There was no effect of the pineal lesion in the LD 6:18 birds. In the LD 12:12, the pineal lesion neither inhibited testicular growth nor significantly augmented testicular growth, a possibility since this 12-hr lighting regimen is not maximally stimulatory in the House Finch (Hamner 1966).

After completing experiment I we had no reason, according to the theory of Wurtman and Axelrod, to expect any further effects. Nevertheless, experiment II was attempted to assess whether the pineal might differentially affect termination of photorefractoriness. Table 2 demonstrates that the presence of a lesioned pineal has no apparent effect on termination of photorefractoriness.

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TABLE 1. Weight (mg) of left testis of pineal lesioned and sham-operated House Finches exposed to two light-dark (LD) regimes for 28 days.

LD 6 : 18		LD 12 : 12	
Sham	Lesion	Sham	Lesion
1.6	2.0	46.1	53.7
1.0	1.6	12.8	47.0
2.3	1.1	37.2	49.1
2.0	1.5	52.5	19.0
0.8	0.9	46.3	26.1
1.3	1.5	20.0	26.4
0.6	0.3	12.5	12.8
0.5	1.8	9.6	29.8
1.8	1.8	9.5	6.3
2.0	0.4	26.4	44.0
	0.5		43.2
$\bar{x} = 1.4$	1.4	$\bar{x} = 27.3$	$\bar{x} = 32.5$
	1.5		
	0.6		
	2.8		
	0.9		
	$\bar{x} = 1.3$		

TABLE 2. Weight (mg) of left testis of pineal lesioned and sham-operated House Finches exposed to two lighting regimens for 65 days, followed by LD 18 : 6 for 21 days.

LD 6 : 18		LD 18 : 6	
Sham	Lesion	Sham	Lesion
35.0	20.5	0.8	0.5
30.0	15.5	1.0	0.8
6.2	31.3	1.0	0.5
	27.5	0.6	0.3
$\bar{x} = 23.7$	20.4		1.2
	19.6	$\bar{x} = 0.9$	1.0
	23.7		0.8
	13.0		1.0
	13.1		0.4
			0.5
	$\bar{x} = 20.5$		1.0
			$\bar{x} = 0.7$

Upon examining the brains, it was apparent that not all of the lesions were completely effective. Approximately 75 per cent of the pineal material in several brains was destroyed. In others, however, all of the pineal tissue appeared to have been destroyed. Even though our lesions did not destroy all of the pineal in some birds, if melatonin actually does inhibit reproductive maturation, we believe that we should have seen at least some effect of these lesions in at least a few birds; yet none was observed.

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PHOTOREFRACTORINESS IN PINEALECTOMIZED HARRIS' SPARROWS

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Exposure to long daily photoperiods induces testicular growth in photosensitive passerine species and, if prolonged, inevitably causes testicular regression. The latter signals the onset of refractoriness which, under natural conditions, is not eliminated without the effect of short daily photoperiods (for reviews see Farner 1959, 1964, 1967; Farner and Follett 1966). Since the pineal body has an antigonadotropic function in the golden hamster (Reiter et al. 1966; Reiter 1967) and perhaps in the rat (Wurtman et al. 1959; Reiter et al. 1968), the possibility exists that this organ plays a role in refractoriness in photoperiodic passerine species. To test this possibility, we examined the effect of pinealectomy on testicular regression in photostimulated Harris' Sparrows (*Zonotrichia querula*) and on testicular quiescence in refractory Harris' Sparrows retained on long daily photoperiods.

Birds captured with mist nets near Manhattan, Kansas, were housed, several per cage, in Hendryx breeding cages. Illumination was provided by fluorescent lamps at an intensity of at least 375 lux. Ambient temperature ranged between 18° and 24°C. At autopsy, testes were removed and fixed for 5 days in AFA; after 5 additional days in 70 per cent ethanol, they were debrided and weighed on a torsion balance. Statistical analysis of testicular weights was by Student's *t*-test. The region of the pineal body was inspected microscopically to verify operational success.

Harris' Sparrows captured between 17 December 1966 and 4 February 1967 were held on 8-hr daily photoperiods (08:30–16:30 CST) until 27 February 1967, when testicular growth was induced by lengthening the daily photoperiod to 20 hr (08:30–04:30 CST). Twenty days later, i.e., near the end of the logarithmic growth phrase (Wilson 1968), pinealectomy or sham pinealectomy (for procedures see Donham 1968) was performed and the birds returned to long daily photoperiods. They were sacrificed 54 or 79 days postoperatively. The data in table 1 show that pinealectomy neither prevented testicular regression nor altered its course. Both pinealectomized and sham-pinealectomized Harris' Sparrows had smaller testes after 99 days than after 74 days of photostimulation. Moreover, testicular weights of pinealectomized birds were not significantly different from those of sham-pinealectomized birds at either killing date.

TABLE 1. Failure of pinealectomy to prevent testicular regression in photostimulated Harris' Sparrows.

Group	Operational procedure[a]	Days on 20-hr daily photoperiods after operation	Testicular weight $\bar{x}$ mg ± SE (*n*)
1	P	54	39.60 ± 8.101 (4)
	S		23.28 ± 5.778 (5)
2	P	79	6.91 ± 0.963 (4)
	S		4.91 ± 0.473 (6)

[a] P = pinealectomy; S = sham pinealectomy. Both were performed on day 20 of exposure to 20-hr daily photoperiods.

In another experiment, male Harris' Sparrows captured between 12 March and 16 April 1966 were held on 13-hr daily photoperiods (08:30–21:30 CST) until 2 May 1966 when the daily photoperiod was increased to 20 hr (08:30–04:30 CST). About 5 months later (during refractoriness), pinealectomy or sham pinealectomy was performed and the birds returned to long days. Birds were killed either 1.5 or 3 months later. As in the previous experiment, pinealectomy did not eliminate photorefractoriness. Testes of pinealectomized and of sham-pinealectomized birds were small at both killing dates (table 2).

Our observations suggest that the pineal body plays no role in the natural termination of reproductive activity in male Harris' Sparrows and, further, that the insensitivity of refractory birds to photoperiodic

TABLE 2. Failure of pinealectomy to induce testicular growth in refractory Harris' Sparrows held on 20-hr daily photoperiods.

Group	Operational procedure[a]	Months on 20-hr daily photoperiods after operation	Testicular weight $\bar{x}$ mg ± SE (*n*)
1	P	1.5	1.97 ± 0.229 (7)
	S		2.10 ± 0.020 (2)
2	P	3	2.30 ± 0.507 (5)
	S		2.45 ± 0.659 (4)

[a] P = pinealectomy; S = sham pinealectomy. Both were performed after approximately 5 months of daily 20-hr photoperiods.

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