THE NEUROSECRETORY SYSTEM AND PHOTOINDUCED TESTICULAR GROWTH IN HARRIS' SPARROW (ZONOTRICHIA QUERULA)

by

JOHN KENNETH BOON

B. A., Houghton College, 1962

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

BIOLOGY

Division of Biology

KANSAS STATE UNIVERSITY Manhattan, Kansas

1970

Approved by:

Major Professor

LD 2668 T4 1969 B66

TABLE OF CONTENTS

INTRODUCTION	1
MATERIALS AND METHODS	3
Experiment I	3
Experiment II	4
RESULTS	6
The Hypothalamic Neurosecretory System of Zonotrichia querula	6
Experiment I	9
Experiment II	11
DISCUSSION	25
ACKNOWLEDGMENTS	31
LITERATURE CITED	32

INTRODUCTION

The neuroendocrine mechanisms that regulate photoperiodic testicular growth in birds have been the subject of intensive research, particularly during the last decade (for review, see Farner et al., 1967). The hypothesis that the paraldehyde-fuchsin-stainable (PAF+) hypothalamic neurosecretory system is an essential component of the mechanism of photoperiodic testicular growth was first proposed by Benoit and Assenmacher (Benoit and Assenmacher, 1953, 1959; Assenmacher, 1958; Benoit, 1962, 1964) using the domestic mallard and later supported by investigations on the White-crowned Sparrow (Oksche et al., 1958, 1959; Farner et al., 1960, 1962; Farner, 1962), the White-throated Sparrow (Wolfson and Kobayashi, 1962), the White-eye (Uemura and Kobayashi, 1963), the Tree Sparrow (Passer montanus saturatus) (Matsui, 1966), and the Japanese Quail (Konishi, 1967). Preceding these investigations were the observations of Wingstrand (1951) which indicated an intimate, possibly functional, relationship between the avian hypothalamus and pars distalis.

Although for the domestic mallard the evidence is convincing, for other avian species there is uncertainty concerning the functional role of PAF + material in the zona externa of the anterior median eminence. Dissimilar responses in White-crowned and White-throated Sparrows during the course of photoinduced testicular growth have prompted a study of the effect of photostimulation on the density of PAF + material in the anterior median eminence of a third species of Zonotrichia, Harris' Sparrow. Further, recent evidence based on ablative techniques suggests that the PAF + fibers that terminate in the anterior median eminence do not comprise the final neural pathway that regulates photoperiodic testicular growth in either the White-

crowned Sparrow (Wilson, 1967; Stetson, 1969) or the Tree Sparrow (Spizella arborea) (Wilson and Hands, 1968). It thus seemed pertinent to study also the effect of surgical lesions in the PAF + neurosecretory tract on the photoperiodic testicular response of Harris' Sparrow.

MATERIALS AND METHODS

Experiment I. The relationship between density of PAF + material in the zona externa of the anterior median eminence and testicular weight was studied in 45 photosensitive Harris' Sparrows. The birds, captured during midwinter and retained for one to two months on 8-hour daily photoperiods, were exposed to 20L-4D (Group PSL) or held on 8L-16D (Group PSS) and sacrificed by decapitation at 5-day intervals over a period of 20 days. [For additional details regarding capture, handling, and experimental design, see Wilson (1968).] Brains were fixed in Bouin's fluid and dissected according to the procedure of Oksche et al. (1959). Frontal sections of hypothalami embedded in Paraplast through methylbenzoate-benzene were stained with paraldehyde-fuchsin.

Insofar as possible, hypothalami from PSS and PSL birds were stained in the same tray (see Table 1). For each bird, density of PAF + material in the zona externa of every fourth section of anterior median eminence was evaluated subjectively and a value from 0 to 5 assigned thereto. The former indicated absence of PAF + material; the latter was assigned to sections having the greatest density. The number of sections assigned to each density class was multiplied by the value of that class and the sum of the products divided by the number of sections evaluated. The quotient, a weighted mean, is the neurosecretory index (Lisk, 1965/1966). Because of the subjectivity in estimating the density, each median eminence was evaluated three times. In the first analysis, median eminences were compared with others from the same tray; in the second, median eminences were compared with members of the same subgroup but, as in the first analysis, without reference to PSS or PSL

identity. In the third analysis, each PSL median eminence was compared with a PSS median eminence from the same tray. From the three analyses an average neurosecretory index was calculated.

Neurosecretory indices were plotted against number of days on long photoperiods and logarithms of testicular weights (Wilson, 1968). Regression lines were determined by the method of least squares and 95 per cent confidence limits applied. The significance of differences between index means of PSS and PSL birds within subgroups was tested by analysis of variance.

Experiment II. The effect of disruption of the hypothalamo-hypophyseal tract on testicular growth was studied in 15 photosensitive Harris' Sparrows. Birds captured on 25 January and 2 February 1968 were retained on 8L-16D until the beginning of the experiment. Birds captured on 10 April 1968 were placed on 20L-4D for 13 weeks and then transferred to 8L-16D for 19 weeks so that they, too, were photosensitive at the beginning of the experiment. At least 20 weeks prior to the experiment the birds were sexed by laparotomy. They were housed in small cages with food, water, illumination and temperature as in Experiment I (Wilson, 1968).

Because of the small sample size and the probability that interruption of the hypothalamo-hypophyseal tract would not be 100 per cent successful, all birds were subjected to surgery. Following induction of anesthesia by pentobarbital sodium (0.07 mg/g body wt.), surgery was performed using the procedure of Wilson and Hands (1968) except that (1) the knife was modified by replacing stainless steel wire with Hastelloy steel which could be ground to provide a sharper blade, and (2) the blade was rotated four times through 360 degrees. Birds were transferred to 20L-4D on the first postoperative day and sacrificed by decapitation 20 days later.

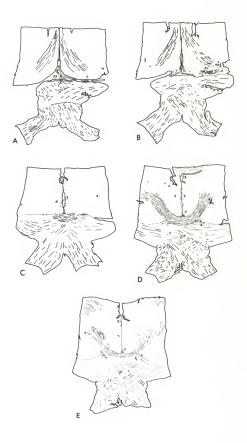
Hypothalami were prepared as in Experiment I except that dioxane was used as intermediate. Frontal sections (10 μ) were stained with paraldehyde-fuchsin and examined for lesions in the hypothalamo-hypophyseal tract and for density of PAF + material in the median eminence. Birds showing no dis-ruption of the tract were classified as sham-operated controls. In lesioned birds the area of the zona externa lacking PAF + material because of the lesion was estimated and expressed as per cent of total area of zona externa of the anterior median eminence.

Testes were fixed in acetic acid, ethanol, and formalin for five days prior to transfer to 70 per cent ethanol. Five days later both testes were weighed to the nearest 0.1 mg on a torsion balance.

RESULTS

The Hypothalamic Neurosecretory System of Zonotrichia querula. The architecture of the hypothalamic neurosecretory system of Zonotrichia querula is fundamentally like that of neurosecretory systems of other avian species (Oksche et al., 1959; Kobayashi et al., 1961; Laws, 1961; Matsui, 1964; Wilson and Hands, 1968). Cell bodies of neurosecretory cells, identified by their PAF + content, are in loosely arranged clusters that are recognized as divisions of the supraoptic and paraventricular nuclei (Figure 1). The median division of the supraoptic nucleus first appears in the roof of the preoptic recess just posterior to the lamina terminalis and extends posteriorly on each side of the third ventricle. The lateral division, surrounding prominent blood vessels as in the White-crowned Sparrow (Oksche et al., 1959), appears just posterior to the rostral margin of the median division and is especially prominent because of its compactness and number of cells. The median and lateral divisions are connected by a thin line of cells that extends laterally along the optic tract and may correspond to the preoptic division described by Laws (1961) in the White-crowned Sparrow. The posterior division of the supraoptic nucleus is rostral to the posterior border of the optic chiasma but posterior to the termini of the lateral and median divisions. Cells of the posterior division are clustered in the optic tract ventral to the level where PAF + material first becomes visible in the hypothalamo-hypophyseal (neurosecretory) tract. The paraventricular nucleus is less compactly arranged than the supraoptic nucleus. Its ventral division begins posterior to the median division of the supraoptic nucleus; its lateral division is located laterally at about the same level. Cells of the ventral

Figure 1. Schematic diagrams showing the divisions of the neurosecretory nuclei in Harris' Sparrow. Frontal sections ABCDE appear in order rostrocaudally. Nuclei are represented by dots. Supraoptic nucleus: 1, median division; 2, lateral division; 6, posterior division. Paraventricular nucleus: 3, ventral division; 4, lateral division; 5, dorsal division. 67, optic tract; PR, preoptic recess; EV, blood vessels; v, 3rd ventricle.



division lie close to the ependyma of the third ventricle and extend along it posterodorsally. The beginning of this division is barely distinguishable from the caudal portion of the median division of the supraoptic nucleus. A few cells composing the dorsal division of the paraventricular nucleus are located posteriorly and dorsally to its ventral division. Cells of these two divisions are often widely separated and are occasionally located some distance from the ependyma.

The remainder of the neurosecretory system consists of fibers that extend from the supraoptic and paraventricular nuclei to the anterior median eminence and to the neural lobe; these fibers are referred to collectively as the hypothalamo-hypophyseal tract. Fibers could not be traced from cell bodies since their proximal ends contain little or no visible PAF + material. Stainable material in the hypothalamo-hypophyseal tract becomes visible just rostral to the median eminence.

The median eminence is composed of an anterior division that contains large amounts of PAF + material in the zona externa and a posterior division that contains PAF + material only in the neurosecretory tract as it passes to the neural lobe. The amount of neurosecretory material along the course of the anterior median eminence varies widely among and within individuals.

The neural lobe arises at the posterior end of the infundibular stem, is thin walled, and very rich in PAF + material.

Experiment I. The data of Table 1 suggest that photostimulation has no effect on the density of PAF + material in the zona externa of the anterior median eminence in Harris' Sparrow. Differences between mean neurosecretory indices of PSS and PSL birds within subgroups, based on intratray and intertray comparisons, were not significant at the 95 per cent level. Thus,

TABLE 1

Effect of 20-Hour Daily Photoperiods on Neurosecretory Index of the Anterior Median Eminence and Left Testicular Weight.

Subgroup	Tray	Group	Neurosecre Average	etory Index Range	Range of Test. Wt. (mg)
O Daya	3 10	PSS (4) ^b PSS (1)	3.1 3.0	2.8-3.3	0.5-0.7 0.8
5 Day	1 2 12	PSS PSL PSS PSL PSS (1) PSL (1)	3.7 3.7 3.1 2.5 3.1 3.9	3.2-4.2 3.4-3.9 - 2.0-3.0	0.6 0.9 0.8-1.8 1.1-1.7 1.4 2.5
10 Day	4 5 13	PSS PSL PSS PSL PSS (1) PSL (1)	3.5 3.8 3.8 2.8 c	3.1-3.9 3.6-3.9 2.7-3.0 2.1-3.5	0.9 2.7-4.1 1.2-1.4 5.0 12.4
15 Day	6 8 12	PSS PSL PSS PSL PSS (1) PSL (1)	3.0 3.6 3.0 2.7 4.2 3.5	2.8–3.1 3.4–3.8 2.5–3.4 2.4–3.0	0.5-1.7 15.0-16.0 1.4 25.1-27.2 0.9 19.0
20 Day	7 9 13	PSS PSL PSS PSL PSS (1) PSL (1)	2.8 3.5 2.9 3.0 2.8 3.8	2.7-2.9 2.9-4.2 2.4-3.5 2.2-3.7	0.7 44.5-48.3 1.4 53.1-56.4 1.1 128.9

a Initial controls.

b Neurosecretory averages are based on 2 individuals unless noted otherwise.

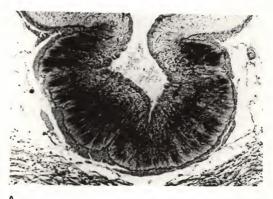
c Not used in the analysis.

birds exposed to stimulatory daily photoperiods for up to 20 days showed no significant accumulation or depletion of PAF + material from the median eminence; neurosecretory indices were comparable in FSS and FSL birds with the former having a range of 2.4-4.2 and the latter, a range of 2.0-4.2. From these ranges (Table 1 and Figure 2) it is apparent that some FSS birds contained less neurosecretory material than others and less than some FSL birds which developed large testes. There was considerable individual variation in both groups, but differences among FSL subgroups were slight. When neurosecretory indices of PSL birds were plotted against number of days! exposure to 20-hour daily photoperiods (Figure 3), the regression coefficient was not significantly different from zero.

Although 20-hour daily photoperiods had no significant effect on the density of PAF + material in the anterior median eminence, PSL birds exhibited testicular growth which during the first 20 days approximated a logarithmic function of time (Wilson, 1968). Left testicular weights of PSL birds reached 44.5-128.9 mg by day 20 compared to 0.7-1.4 mg in PSS birds and 0.5-0.8 in initial controls (Table 1). When neurosecretory index was plotted against logarithm of testicular weight (Figure 4) the regression line had a positive slope that was not significantly different from zero. Testicular growth, therefore, was not correlated with a change in density of PAF + material in the zona externa of the anterior median eminence.

Experiment II. With one possible exception, partial deafferentation of the median eminence did not curtail the photoperiodic testicular response of Zonotrichia querula. Of the 15 males subjected to surgery, lesions were found in the hypothalamo-hypophyseal tracts of seven at the rostral part of the anterior median eminence. Of these, five contained lesions which

Figure 2. Frontal sections of anterior median eminences of two male Harris' Sparrows showing relative amounts of PAF + material. a) PSL bird, 20 days. Left testicular weight = 128.9 mg. Neurosecretory index = 3.8. b) PSS bird, 20 days. Left testicular weight = 1.4 mg. Neurosecretory index = 3.5. Paraldehyde-fuchsin. 388%.



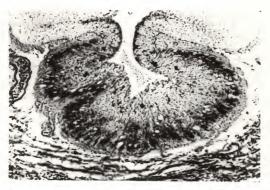


Figure 3. Effect of 20-hour daily photoperiods on the neurosecretory index of the anterior median eminence during the logarithmic phase of testicular growth. Eroken lines indicate 95 per cent confidence limits.

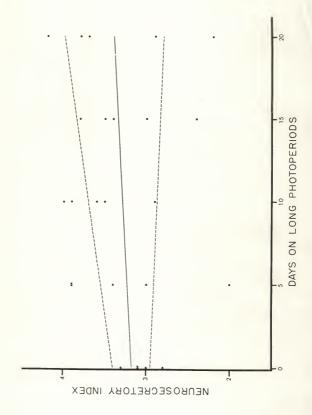
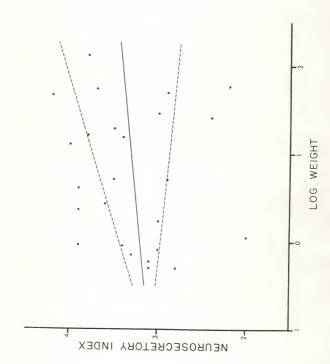


Figure 4. Relationship between neurosecretory index and logarithm of left testicular weight in birds exposed to 20-hour daily photoperiods for 0 to 20 days. Broken lines indicate 95 per cent confidence limits.



considerably reduced the amount of PAF + material in the median eminence (Table 2). Figure 5 illustrates for three of the five birds the lesions and their effects on the density of PAF + material in the median eminence. Two birds (0360 and 0367) contained lesions which disrupted a small portion of the tract and part of the palisade layer and pars tuberalis but resulted in the smallest reduction of PAF + fibers (Table 2). In these, intact areas of anterior median eminence resembled the same areas in some sham-operated birds. In sham-operated birds, the neurosecretory system appeared unaffected by lesions and the anterior median eminence contained small to large quantities of evenly distributed PAF + material. Figure 6 illustrates dense accumulation of PAF + material in the zona externa of a sham-operated bird which developed large testes. Some derangement was detected in the optic chiasma in a few sham-operated birds but the neurosecretory tract and median eminence were unaffected. In none of the birds was there evidence of surgical insult to the infundibular nucleus.

Although testes of both lesioned and sham-operated birds responded to 20-hour daily photoperiods the testicular response was minimal in lesioned bird 0360 and possibly reduced in bird 0367. In both birds the pars tuberalis and palisade layer were partially disrupted but the neurosecretory tract rostral to the median eminence was only slightly damaged. Testicular growth was also reduced in sham-operated birds 0365 and 0368. Lesions in these birds barely contacted the hypothalamo-hypophyseal tract and were considered inconsequential because there was no obvious decrease in density of PAF + material in the median eminence. Testes of other sham-operated and lesioned birds approached or, in some cases, exceeded the weights reported for intact adult and immature males exposed to 20-hour photoperiods for 20 days (Wilson, 1968). At autopsy, the anterior median eminences of sham-operated birds

TABLE 2

Effect of Lesions in the Rostral Portion of the Anterior Median Dminence on Testicular Weight in Photosemsitive, Photostimulated Harris' Sparrows Sacrificed 21 Days After Surgery.

Bird No.	Group	Per cent of zona externa lacking intact PAF + fibers	Weight of both testes (mg)	
0336	So	0	422.0	
0340	So	0	582.8	
0341	So	0 .	456.1	
0348	So	0	586.5	
0353	So	0	178.3	
0359	So	0	164.2	
0365	So	0	80.1	
0368	So	0	47.7	
0338	L	82	232.9	
0352	L	87	410.3	
0360	L	78	18.7	
0367	L	82	68.4	
0370	L	84	132.8	
0371	L	88	257.6	
0373	L	91	192.7	

So = Sham-operated control

L = Lesioned bird

Figure 5. Effect of lesions (†) in the rostral part of the AME on the density of PAF + material. A) 0371. Lesion. B) 0371. AME showing density of PAF + material. Weight of both testes = 257.6 mg. C) 0373. Lesion. D) 0373. AME. Weight of both testes = 197.7 mg. E) 0352. Lesion. F) 0352. AME. Weight of both testes = 410.3 mg. Paraldehyde-fuchsin. 215X.

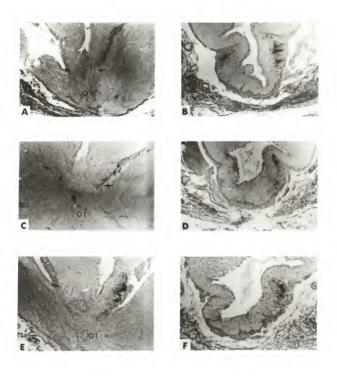
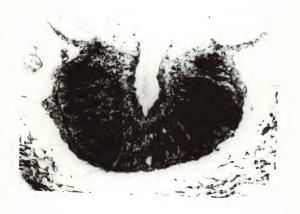


Figure 6. 0336. Frontal section of AME of sham-operated bird exposed to 20-hour daily photoperiods for 20 days showing very dense PAF + material. Weight of both testes = 422.0 mg. Paraldehyde-fuchsin. 350%.



contained light to heavy amounts of PAF + material. In birds sustaining lesions in the hypothalamo-hypophyseal tract, the testes also developed extensively (132.8-410.3 mg). Although some of the sham-operated birds developed larger testes (47.7-586.5), the range of testicular weights of lesioned birds (except 0360) lay well within that of the sham-operated group. Testicular growth was apparent in the two birds in which the palisade layer was disturbed (0360 and 0367) but was not as extensive as in the others (Table 2).

None of the lesions totally transected the hypothalamo-hypophyseal tract. However, in lesioned birds, only a small portion (9-22 per cent) of the anterior median eminence contained intact PAF + fibers (Figure 5 and Table 2). Even so the testes responded to long daily photoperiods as they did in sham-operated controls.

The results suggest that impairment of the hypothalamo-hypophyseal tract by surgical lesions in the most rostral portion of the anterior median eminence does not adversely affect the photoperiodic testicular response of Harris' Sparrow.

DISCUSSION

The results of Experiment I indicate that the density of PAF + material in the zona externa of the anterior median eminence did not change appreciably throughout the 20-day period of exposure to 20-hour daily photoperiods. Moreover, examinations at 5-day intervals revealed comparable amounts of PAF + material in the median eminences of PSL and PSS birds (Table 1) even though testicular growth was induced in the former but not in the latter (Wilson, 1968; see also Table 1). These findings, though not necessarily negative, do not strengthen the hypothesis that the eminential component of the hypothalamic neurosecretory system is an essential element of the mechanism that controls photoperiodic testicular growth in birds. That hypothesis, first formulated by Benoit and Assenmacher (1953, 1959) and Assenmacher (1958) and based on convincing evidence from intrahypothalamic lesions in the domestic mallard, has also been proposed to account for photoperiodic testicular growth in the White-crowned Sparrow (Zonotrichia leucophrys gambelii) by Oksche et al. (1958, 1959), in the White-throated Sparrow (Zonotrichia albicollis) by Wolfson and Kobayashi (1962), and in the Tree Sparrow (Passer montanus saturatus) by Matsui (1966). In the Whitecrowned Sparrow (Oksche et al., 1958; Farner et al., 1960, 1962) and the Tree Sparrow (Matsui, 1966), depletion of PAF + material from the anterior median eminence in photosensitive, photostimulated birds was interpreted as evidence for involvement of the eminential component of the neurosecretory system in the mechanism of photoinduced testicular growth. On the other hand, Wolfson and Kobayashi (1962) observed an initial increase in the density of PAF + material in the White-throated Sparrow concomitant with photoinduced testic-

ular growth as did Uemura and Kobayashi (1963) in the White-eve and Wolfson (1966) in the Slate-colored Junco. Although Oksche et al. (1964) and Follett and Farmer (1966) observed no appreciable change in density of stainable material in the median eminence of photosensitive, photostimulated Japanese Quail, Konishi (1967) reported that Japanese Quail that developed large testes in continuous light had less PAF + material in the median eminence than those showing no testicular growth after exposure to 8-hour daily photoperiods. In spite of their differences these observations on the Whitecrowned Sparrow, Tree Sparrow, White-throated Sparrow, White-eye, Slatecolored Junco and Konishi's observations on Japanese Quail have been interpreted as evidence that the eminential component of the hypothalamic neurosecretory system participates in the control of the adenohypophysis which in turn regulates testicular growth. Although there was depletion of PAF + material in the White-crowned Sparrow (Farner, 1962) and accumulation in the White-throated Sparrow (Wolfson and Kobayashi, 1962) there was an increase in acid-phosphatase activity in the median eminence in both species during photoinduced testicular development. Acid-phosphatase activity was thought to be related to the function of the neurosecretory system since Kobayashi et al. (1962b) showed that release of stainable material from the pars nervosa in dehydrated rats and pigeons is accompanied by an increase in acidphosphatase activity, thus giving credibility to the hypothesis that increased phosphatase activity reflects increased release of the neurohormone from the axons of the eminential component of the hypothalamic neurosecretory system. Moreover, increased proteinase activity in the median eminence of the White-crowned Sparrow during photoinduced testicular development (Kobayashi et al., 1962a) was interpreted as additional support for the

hypothesis that neurohormone is released from the hypothalamo-hypophyseal neurosecretory system. However, it is plausible that activity of both enzymes in the median eminence may reflect the functional state of the paraldehyde-fuchsin-negative (PAF -) fibers which are present and have been implicated in the control of testicular growth by Wilson (1967) and Stetson (1969). There is no clear-cut evidence from Experiment I of this study that PAF + material is involved in the photoperiodic testicular response since there is no visible sign of accumulation or depletion of stainable material from the anterior median eminence. A recent investigation on the density of stainable material in the median eminence of photosensitive, photostimulated White-crowned Sparrows (Bern et al., 1966) as well as investigations of Oksche et al. (1964) and of Follett and Farner (1966) on Japanese Quail have produced results similar to those reported here. The conclusion that the density of PAF + material in Harris' Sparrow is not related to the control of testicular growth by the adenohypophysis is consistent with the report of Graber and Nalbandov (1965) that neither increased daily photoperiods nor adenohypophysectomy had any noticeable effect on the density of neurosecretory material in the median eminence of the cockerel. Further, Graber et al. (1967) reported that there was no correlation between density of neurosecretory material and testicular growth in cockerels bearing lesions in the hypothalamo-hypophyseal tract.

The available evidence does not lend itself to a simple conclusion about the effect of long daily photoperiods on the density of PAF + material in the median eminence in different species of birds. It is reasonable that, if a neurohormone is released from the anterior median eminence under the influence of long daily photoperiods, specific differences in PAF + content

may reflect different ratios of rates of synthesis and release of the material. Although the results of Experiment I led to the cautious conclusion that PAF + material is not released during photoinduced testicular growth and therefore is not the factor responsible for release of testicular growthstimulating hormone from the adenohypophysis, interruption of the PAF + hypothalamo-hypophyseal tract seemed to be a more direct approach to the assessment of the role of PAF + material in the mechanism of photoperiodic testicular growth. In Experiment II of this study, lesions in the hypothalamo-hypophyseal tract in five Harris' Sparrows did not alter the photoperiodic testicular response even though at autopsy their median eminences were nearly devoid of PAF + material as a result of the lesions. Lesioned birds retained a few intact fibers of the hypothalamo-hypophyseal tract (Figure 5) and these contained dense PAF + material. Since all fibers were not interrupted, some restraint in interpretation must be employed. Although it cannot be stated without reservation that the PAF + material has no function in the photoperiodic testicular response, consideration of the enormous number of fibers in sham-operated birds makes it seem unlikely that the few fibers that remained intact in lesioned birds could have supported the degree of testicular growth achieved by the twentieth day of photostimulation. In light of the degree of testicular growth that occurred in birds sustaining extensive damage to the hypothalamo-hypophyseal tract it is difficult to assess the testicular response in bird 0360 wherein the palisade layer and pars tuberalis were disrupted without extensive damage to the neurosecretory tract. Testicular weight in a second bird (0367) that received similar damage was within the range of testicular weights in sham-operated controls. Testicular growth in lesioned Harris' Sparrows, quantitatively similar to

that in sham-operated controls, strongly suggests that the photoperiodic testicular response is independent of the PAF + neurosecretory system.

Wilson (1967) demonstrated that electrolytic lesions involving the entire median eminence or the infundibular nuclear region (in the latter case the anterior median eminence retained a nearly full complement of PAF + fibers) abolished or markedly suppressed the photoperiodic testicular response of the White-crowned Sparrow but lesions in the anterior median eminence alone did not. Similar work by Stetson (1969) in the same species showed that lesions that destroyed the anterior median eminence alone or interrupted the hypothalamo-hypophyseal tract rostral to the median eminence. thereby causing marked depletion of PAF + material from the median eminence. generally had no effect on testicular growth while lesions that destroyed the posterior median eminence abolished testicular growth and lesions that destroyed portions of the infundibular nucleus suppressed the photoperiodic testicular response. The data of Wilson (1967) and Stetson (1969) support the hypothesis that, in the White-crowned Sparrow, the PAF - infundibular neuron system rather than the PAF + neurosecretory system is an essential part of the mechanism that controls photoperiodic testicular growth. Surgical lesions that interrupted the hypothalamo-hypophyseal tract and caused disappearance of PAF + material from the median eminence of the Tree Sparrow (Spizella arborea) (Wilson and Hands, 1968) had no apparent effect on the photoperiodic testicular response. Electrolytic lesions in the region of the neurosecretory nuclei caused cessation of reproductive function in chickens (Ralph, 1959; Ralph and Fraps, 1959; Egge and Chiasson, 1963) but more recent work indicated that hypothalamic lesions that affected the antidiuretic mechanism did not prevent reproductive function as long as the damaged area

excluded the stalk (Koike and Lepkovsky, 1967), and that lesions of the ventromedial nucleus that impaired the release of gonadotropins and caused hyperphagia (Lepkovsky and Yasuda, 1966) had not damaged components of the neurosecretory system. Much of the current evidence provided by ablation or partial interruption of the neurosecretory system fails to show that PAF + material is responsible for the control of testicular growth.

The foregoing observations on Harris' Sparrow indicate that there is no appreciable effect of photostimulation on the density of PAF + material in the zona externa of the anterior median eminence and, from the effect of surgical lesions in the hypothalamo-hypophyseal tract, that PAF + material probably is not a vital factor in the mechanism of photoperiodic testicular growth. Rather, they tend to support the hypothesis, based on intrahypo-thalamic lesions in the chicken (Lepkovsky and Yasuda, 1966; Koike and Lepkovsky, 1967; Graber et al., 1967), White-crowned Sparrow (Wilson, 1967; Stetson, 1969), and Tree Sparrow (Wilson and Hands, 1968), that a factor other than PAF + material affects photoinduced release of growth-stimulating gonadotropin(s) from the adenohypophysis.

ACKNOWLEDGMENTS

The assistance I have received from several individuals in the course of this project has been appreciated and deserves mention.

I express my appreciation in particular to Dr. Fred E. Wilson for his direction in the experimental work and in the preparation of this thesis. Professors D. J. Ameel, M. R. Fedde, H. T. Gier and L. E. Roth have critically reviewed the manuscript.

I thank Dr. H. T. Gier for his help in photographing the material illustrated herein.

I appreciate the suggestions of B. H. Bailey and J. H. Tormey in selecting a material suitable for constructing the knife and the generosity of William C. Cundiff of Kentucky Electronics, Inc., Owensboro, Kentucky, for supplying specially drawn wire of that material.

Mr. Richard S. Donham deserves acknowledgment for his efforts in collecting Harris' Sparrows.

Finally, I wish to thank my wife, Ruth, for her support and assistance in the preparation of this thesis.

LITERATURE CITED

- Assenmacher, I. 1958. Recherches sur le contrôle hypothalamique de la fonction gonadotrope préhypophysaire chez le canard. Arch. Anat. micr. Morph. exp., 47:447-572.
- Benoit, J. 1962. Hypothalamo-hypophyseal control of the sexual activity in birds. Gen. Comp. Endocrinol., Suppl. 1:254-274.
- Benoit, J. 1964. The structural components of the hypothalamo-hypophyseal pathway with particular reference to photostimulation of the gonads in birds. Ann. N. Y. Acad. Sci., 117:23-34.
- Benoit, J. et I. Assemmacher. 1953. Rapport entre la stimulation sexuelle préphyophysaire et la neurosécrétion chez l'oiseau. Arch. Anat. micr. Morph. exp., 42:334-386.
- Benoit, J. and I. Assenmacher. 1959. The control by visible radiations of the gonadotropic activity of the duck hypophysis. Recent Progr. Hormone Res., 15:143-164.
- Bern, H. A., R. S. Nishioka, L. R. Mewaldt, and D. S. Farner. 1966. Photoperiodic and osmotic influences on the ultrastructure of the hypothalamic neurosecretory system of the White-crowned Sparrow, Zonotrichia leucophrys gambelii. Z. Zellforsch., 69:198-227.
- Egge, A. S. and R. B. Chiasson. 1963. Endocrine effects of diencephalic lesions in the White Leghorn hen. Gen. Comp. Endocrinol., 3:346-361.
- Farner, D. S. 1962. Hypothalamic neurosecretion and phosphatase activity in relation to the photoperiodic control of the testicular cycle of Zonotrichia leucophrys gambelii. Gen. Comp. Endocrinol., Suppl. 1:160-167.
- Farner, D. S., A. Oksche, H. Kobayashi, and D. F. Laws. 1960. Hypothalamic neurosecretion in the photoperiodic testicular response in birds. Anat. Rec., 137:354.
- Farner, D. S., A. Oksche, and L. Lorenzen. 1962. Hypothalamic neurosecretion and the photoperiodic testicular response in the White-crowned Sparrow, Zonotrichia leucophrys gambelli. Mem. Soc. Endocrinol., 12:187-197.
- Farner, D. S., F. E. Wilson, and A. Oksche. 1967. Neuroendocrine mechanisms in birds. Pp. 529-582 in Neuroendocrinology, Vol. II (L. Martini and W. F. Ganong, ed.), New York, Academic Press.
- Follett, E. K. and D. S. Farner. 1966. The effects of the daily photoperiod on gonadal growth, neurohypophysial hormone content, and neurosecretion in the hypothalamo-hypophysial system of the Japanese Quail (Coturnix coturnix japonica). Gen. Comp. Endocrinol., 7:111-124.

- Graber, J. W., A. I. Frankel, and A. V. Nalbandov. 1967. Hypothalamic center influencing the release of LH in the cockerel. Gen. Comp. Endocrinol., 9:187-192.
- Graber, J. W. and A. V. Nalbandov. 1965. Neurosecretion in the White Leghorn cockerel. Gen. Comp. Endocrinol., 5:485-492.
- Kobayashi, H., H. A. Bern, R. S. Nishioka, and Y. Ryodo. 1961. The hypothalamo-hypophyseal neurosecretory system of the parakeet, Melopsittacus undulatus. Gen. Comp. Endocrinol., 1:545-564.
- Kobayashi, H., S. Kambara, S. Kawashima, and D. S. Farner. 1962a. The effect of photoperiodic stimulation on proteinase activity in the hypothalano-hypophysial system of the White-crowned Sparrow, Zonotrichia leucophrys gambelli. Gen. Comp. Endocrinol., 2:296-310.
- Kobayashi, H., Y. Oota, and T. Hirano. 1962b. Acid phosphatase activity of the hypothalamo-hypophyseal system of dehydrated rats and pigeons in relation to neurosecretion. Gen. Comp. Endocrinol., 2:495-498.
- Koike, T. and S. Lepkovsky. 1967. Hypothalamic lesions producing polyuria in chickens. Gen. Comp. Endocrinol., 8:397-402.
- Konishi, T. 1967. Neurosecretory activities in the anterior median eminence in relation to photoperiodic testicular responses in young Japanese Quail (<u>Coturnix</u> <u>coturnix</u> <u>japonica</u>). Endocrinol. Jap., 14:60-68.
- Laws, D. F. 1961. Hypothalamic neurosecretion in the refractory and postrefractory periods and its relationship to the rate of photoperiodically induced testicular growth in Zonotrichia leucophrys gambelli. Z. Zellforsch., 54:275-306.
- Lepkovsky, S. and M. Yasuda. 1966. Hypothalamic lesions, growth and body composition of male chickens. Poultry Sci., 45:582-588.
- Lisk, R. D. 1965/1966. Neurosecretion in the rat: changes following neural implant of estrogen. Neuroendocrinology, 1:83-92.
- Matsui, T. 1966. Effect of prolonged daily photoperiods on the hypothalamic neurosecretory system of the Tree Sparrow (<u>Passer montanus saturatus</u>). Endocrinol. Jap., 13:23-28.
- Oksche, A., D. F. Laws, and D. S. Farner. 1958. The daily photoperiod and neurosecretion in birds. Anat. Rec., 130:433.
- Oksche, A., D. F. Laws, F. I. Kamemoto, and D. S. Farner. 1959. The hypothalamo-hypophysial neurosecretory system of the White-crowned Sparrow, Zonotrichia leucophrys gambelli. 2. Zellforsch., 51:1-42.
- Oksche, A., W. O. Wilson, and D. S. Farner. 1964. The hypothalamic neurosecretory system of <u>Coturnix coturnix japonica</u>. Z. Zellforsch., 61:688-709.

- Ralph, C. L. 1959. Some effects of hypothalamic lesions on gonadotrophin release in the hen. Anat. Rec., 134:411-428.
- Ralph, C. L. and R. M. Fraps. 1959. Long term effects of diencephalic lesions on the ovary of the hen. Am. J. Physiol., 197:1279-1283.
- Stetson, N. H. 1969. The role of the median eminence in control of photo-periodically induced testicular growth in the White-crowned Sparrow, Zonotrichia leucophrys gambolii. Z. Zellforsch., 23:369-394.
- Uemura, H. and H. Kobayashi. 1963. Effects of prolonged daily photoperiods and estrogen on the hypothalamic neurosecretory system of the passerine bird, <u>Zosterops palpebrosa japonica</u>. Gen. Comp. Endocrinol., 3:253-264.
- Wilson, F. E. 1967. The tubero-infundibular neuron system: a component of the photoperiodic control mechanism of the White-crowned Sparrow, <u>Zonotrichia leucophrys gambelli</u>. Z. Zellforsch., 82:1-24.
- Wilson, F. E. 1968. Testicular growth in Harris' Sparrow (Zonotrichia querula). Auk, 85:410-415.
- Wilson, F. E. and G. R. Hands. 1968. Hypothalamic neurosecretion and photoinduced testicular growth in the Tree Sparrow, <u>Spizella arborea</u>. Z. Zellforsch., 82:303-319.
- Wingstrand, K. G. 1951. The structure and development of the avian pituitary. Lund:Gleerup.
- Wolfson, A. 1966. Environmental and neuroendocrine regulation of annual gondal cycles and migratory behavior in birds. Recent Progr. Hormone Res., 22:177-244.
- Wolfson, A. and H. Kobayashi. 1962. Phosphatase activity and neurosecretion in the hypothalamo-hypophyseal system in relation to the photoperiodic gonadal response in <u>Zonotrichia albicollis</u>. Gen. Comp. Endocrinol., Suppl. 1:168-179.

THE NEUROSECRETORY SYSTEM AND PHOTOINDUCED TESTICULAR GROWTH IN HARRIS' SPARROW (ZONOTRICHIA QUERULA)

by

JOHN KENNETH BOON

B. A., Houghton College, 1962

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

BIOLOGY

Division of Biology

KANSAS STATE UNIVERSITY Manhattan, Kansas Although earlier reports suggested that the paraldehyde-fuchsinstainable (PAF +) hypothalamic neurosecretory system is an essential part of
the mechanism that controls photoperiodic testicular growth in birds, recent
studies based on ablative techniques deny an important role to that system
in the photoperiodic testicular responses of two passerine species. This
recent evidence as well as variable effects of photostimulation on the density
of PAF + material in the median eminence of several passerine species prompted
an investigation on the role of the eminential component of the neurosecretory
system in the photoperiodic testicular response of Harris' Sparrow (Zonotrichia
querula).

Photosensitive intact Harris' Sparrows and Harris' Sparrows with surgical lesions in the most rostral portion of the anterior median eminence were exposed to 20-hour daily photoperiods for 20 days. Examinations at five-day intervals revealed that the density of PAF + material in the anterior median eminence of intact birds was not markedly different from that in the anterior median eminence of nonphotostimulated controls even though testes of photostimulated birds underwent considerable enlargement. In birds sustaining lesions in the hypothalamo-hypophyseal tract, PAF + material was largely absent from the anterior median eminence at autopsy; with one possible exception, the photoperiodic testicular response was unaffected. These observations suggest that neither the integrity of the eminential component of the hypothalamic neurosecretory system nor the PAF + material therein is essential for photoinduced testicular growth in Harris' Sparrow.