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Blockade of Photoperiodically Induced Testicular Growth by Hypothalamic Deafferentation in Japanese Quail (*Coturnix coturnix japonica*)

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Complete deafferentation of the medial basal hypothalamus blocked testicular growth or induced testicular atrophy (dysfunction) in photostimulated Japanese quail. PAS-positive (PAS^+) basophil cells of the adenohypophysis were inactive or regressed. Frontal half-cuts induced the same results but rear half-cuts were ineffective. These results indicate that the neural stimuli evoked by long daily photoperiods are transferred from other brain area(s) to the medial basal hypothalamus through its anterodorsal border. The isolated region includes the nucleus tuberis, which is probably the source of gonadotropin-releasing factor. Adrenals and thyroids were maintained after complete deafferentation of the medial basal hypothalamus.

Recently, hypothalamic control of the pituitary–gonadal axis has been extensively explored in birds (for review, see Farner *et al.*, 1967; Dodd *et al.*, 1971; Kobayashi and Wada, 1973). The ventral hypothalamus is involved in photostimulated testicular growth and maintenance of gonadal activity in white-crowned sparrows, cockerels, tree sparrows, and Japanese quail (Wilson, 1967, 1970; Graber *et al.*, 1967; Sharp and Follett, 1969; Stetson, 1969, 1972a–d; Wada, 1972, 1974; Cusick and Wilson, 1972; Sharp, 1972; Ravona *et al.*, 1973a,b). However, it is not known whether the ventral hypothalamus functions autonomously or is controlled by other brain regions.

In rats, the medial basal hypothalamus was isolated by a small knife from other brain areas, and it was found that phasic gonadotropin secretion was regulated by the preoptic anterior hypothalamic areas, but tonic gonadotropin secretion was controlled by autonomous activity of the medial basal hypothalamus (Halász and Pupp, 1965; Halász and Gorski, 1967; Volschin *et al.*, 1968; Köves and Halász, 1970). In these experiments, gonadotropin secretion was evaluated by observing ovarian and testicular histology. However, when levels of circulating FSH, LH, and prolactin were measured by radioimmunoassay, it was evident that tonic gonadotropin secretion also was depressed after complete deafferentation (Blake *et al.*, 1972, 1973). In the amphibian, *Rana temporaria*, a series of experiments (Dierickx, 1964–1967) showed that the hypothalamic gonadotropic center (pars ventralis tuberis) is not subject to higher neural control but is largely autonomous for gametogenesis and seasonal development of gonads; information from the preoptic area to the gonadotropic center is required for ovulation. In avian species, Wilson and Hands (1968) cut the aldehyde-fuchsin positive (AF^+) supraoptico–hypophyseal tract just behind the optic chiasma in the American tree sparrow, *Spizella arborea*, and observed no effect on photostimulated testicular growth. However, no

experiment such as complete deafferentation of the medial basal hypotalamus in birds has been done.

The present experiments on deafferentation were carried out to determine whether the avian medial hypothalamus is controlled by other brain areas, especially in connection with photoperiodic stimulation of gonadal development.

MATERIALS AND METHODS

Male Japanese quail (*Coturnix coturnix japonica*) were obtained from a commerical source at the age of 3 wk and kept under a short daily photoperiod of 8L 16D (light from 0800 to 1600) for 2–3 wk before transfer to a long daily photoperiod of 16L 8D (light from 0800 to 2400). They were divided into three groups. Group 1 was subjected to hypothalamic deafferentation on the day of transfer to long days. Group 2 was operated after 2-wk photostimulation, and Group 3 was deafferentated after 4-wk photostimulation. Each group had sham-operated controls. The birds were kept on commercial quail food and water. They were killed 2 wk after the operation.

A knife for the hypothalamic deafferentation was made as follows: One end of a stainless steel wire (0.6 mm diam) was sharpened to form a double-edged knife. Then the knife was bent in the shape of a bayonet (Fig. 1a). This knife was inserted into a stainless steel tube (20 gauge) and the other end was bent to form a handle to turn easily. This handle also indicates the angular position of the knife (Halász and Pupp, 1965).

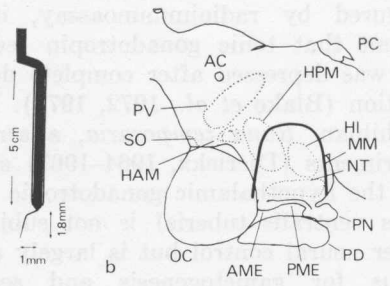

FIG. 1. Schematic drawings of (a) the knife assembly, and (b) the area isolated by the knife cut (thick line). AC, anterior commissure; AME, anterior median eminence; HAM, nucleus hypothalamicus anterior medialis; HI, nucleus hypothalamicus inferior; HPM, nucleus hypothalamicus posterior medialis; MM, nucleus mammillalis medialis; NT, nucleus tuberis; OC, optic chiasma; PC, posterior commissure; PD, pars distalis; PME, posterior median eminence; PN, pars nervosa; PV, nucleus paraventricularis; SO, nucleus supraopticus.

The knife assembly was attached to a stereotaxic apparatus. A bird was anesthetized with Nembutal and fixed on a stereotaxic apparatus in such a position that the median eminence was nearly horizontal (Wada, 1972). The knife was inserted through a hole in the skull made by a dental drill. The blade of the knife was on the median plane while being lowered. An X-ray exposure was taken from the side and the desired level of the knife was attained by referring to the radiograph. Then the knife was turned once 360 degrees (complete deafferentation, CD). The knife assembly was raised slowly and head skin was sutured. Serial sections of the hypothalamus of CD bird killed after 2 wk are shown in Fig. 2. The deafferentated area includes the nucleus tuberis, nucleus hypothalamicus inferior, nucleus mammilaris medialis, and a ventral part of the nucleus hypothalamicus posterior medialis (Fig. 1b) (van Tienhoven and Juhász, 1962); Oksche *et al.* (1972) designated all these nuclei the infundibular nuclear complex as a whole. In subgroups of Group 1, the knife was turned anteriorly and, after insertion, rotated only 90 degrees to the right and left of midline (frontal half-cut, FC) or the knife was turned posteriorly and rotated only 90 degrees to the right and left (rear half-cut, RC). Sham-operated control birds were subjected to the same procedures without rotating the knife.

Body weight and cloacal protrusion were noted every other day during the experiments. Two weeks after operation, the birds of each group were killed by decapitation. The hypothalamus was fixed in Bouin's solution, embedded in paraffin, sectioned serially at 10 μm, and stained with aldehyde fuchsin and toluidine blue O (Asai *et al.*, 1969). Only those birds in which deafferentation was verified histologically are included in the results. The testes, adrenals, thyroids, and adenohypophyses were weighed on a torsion balance and then fixed in Bouin's solution, embedded in paraffin, and sectioned at 6 μm. The testes, adrenals, and thyroids were stained with hematoxylin and eosin. The adenohypophyses were stained with periodic acid-Schiff (PAS), alcian blue, and orange G. The gonadotropic activity of each bird was assessed on the basis of histological observations of the adenohypophysis and testes, and the degree of development of the cloacal protrusion.

RESULTS

The mortality rate after CD was about 65%. If the knife position was too low,

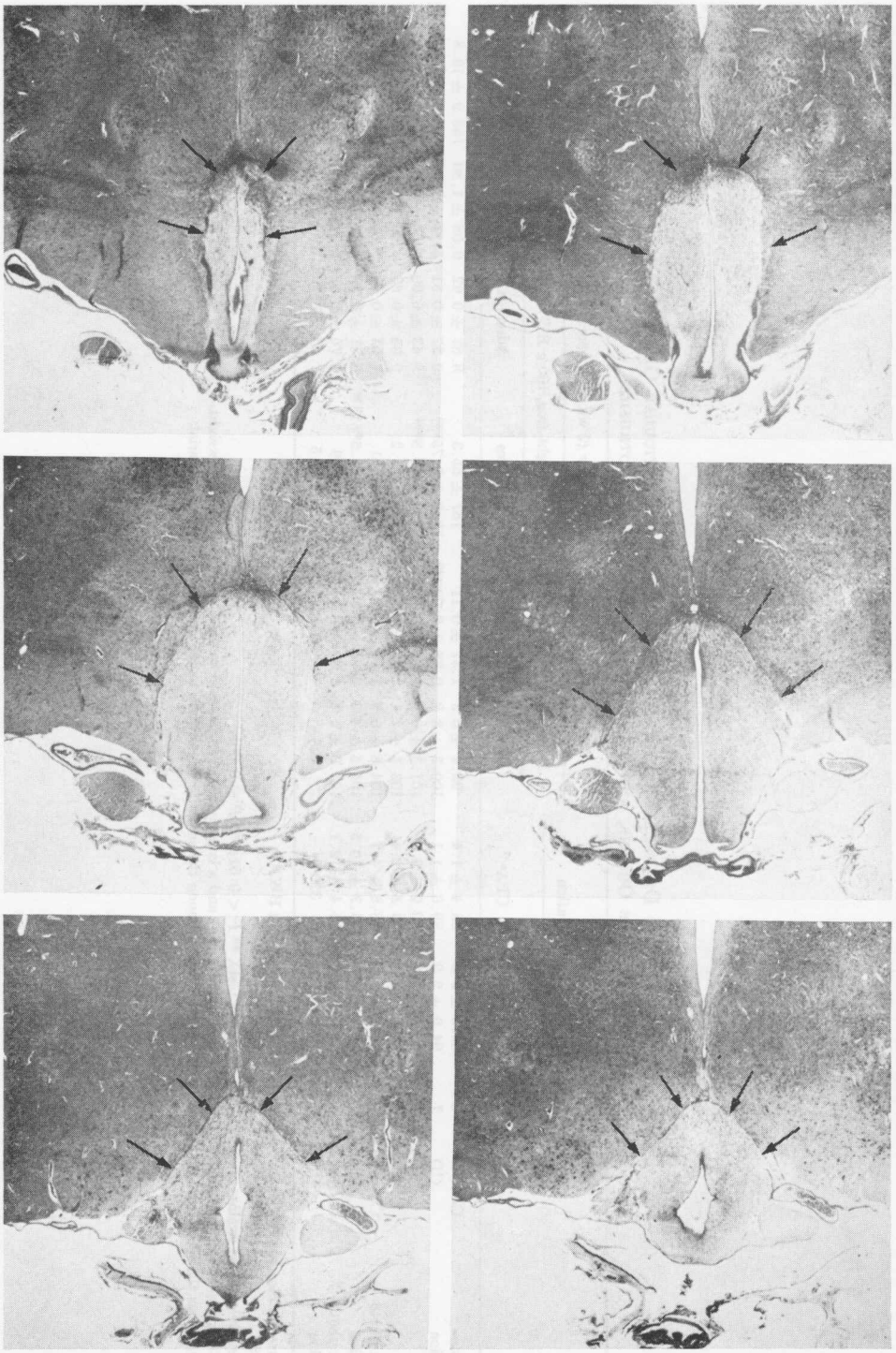

FIG. 2. A series of sections of a hypothalamus 2 wk after complete deafferentation. Arrows indicate the cut. AF-toluidine blue O. ×18.

TABLE 1

EFFECT OF HYPOTHALAMIC DEAFFERENTATION ON PHOTOSTIMULATED TESTICULAR GROWTH, OTHER ENDOCRINE ORGANS, AND DEVELOPMENT OF CLOACAL PROTRUSION

Group		n	Day of operation: Body weight (g)	Day of operation: CPA[a]	At autopsy (2 wk after operation): Body weight (g)	Organ weight (mg/100 g BW): Adenohypophysis	Testes	Adrenals	Thyroids	CPA
1 Before photostimulation	Control	8	89.6 ± 5.3[b]	33.4 ± 1.4	99.4 ± 5.3	1.37 ± 0.11	767 ± 65.3	8.63 ± 0.92	9.08 ± 1.80	158.9 ± 13.8
	CD	7	94.9 ± 2.0	39.0 ± 1.1	100.3 ± 3.2	0.78 ± 0.02***	13.2 ± 0.75***	9.27 ± 0.81	5.85 ± 0.79	36.2 ± 1.85***
	FC	4	95.3 ± 4.2	40.9 ± 2.3	101.3 ± 6.4	1.08 ± 0.08	62.9 ± 43.8***	8.43 ± 0.90	6.54 ± 0.52	37.2 ± 2.99***
	RC	5	95.2 ± 4.1	34.5 ± 2.4	103.4 ± 2.1	1.45 ± 0.14	808 ± 91.2	9.95 ± 0.63	8.40 ± 0.60	165.5 ± 15.9
2 After 2 wk photostimulation	Control	4	102.8 ± 1.1	178.8 ± 4.2	104.8 ± 0.9	1.19 ± 0.04	2980 ± 441	7.63 ± 0.80	7.66 ± 1.04	278.4 ± 10.4
	CD	4	100.8 ± 5.0	154.2 ± 10.5	104.5 ± 4.3	0.85 ± 0.03***	122.8 ± 22.6** (3)[d]	6.81 ± 0.39	7.09 ± 0.68	50.5 ± 3.8***
3 After 4 wk photostimulation	Control	3	99.0 ± 4.6	315.4 ± 12.1	103.0 ± 5.5	1.33 ± 0.14	2880 ± 199	6.76 ± 0.65	7.21 ± 1.96	300.4 ± 8.4
	CD	1	110	320	128	0.96	110.5	6.50	2.62	66.4

[a] CPA = area of cloacal protrusion which is expressed in mm²/100 g BW.

[b] Mean ± SE.

[c] Differences were tested against control of each group (t test). ** $P < 0.01$; *** $P < 0.001$.

[d] In this CD group one bird which had a small right testes (67 mg) and a large left testes (980 mg) was excluded for testicular weight. The heavy testis was hard, and histological observation revealed that the whole testis was filled with numerous cells among the seminiferous tubules which contained colloidal material.

mortality was very high. In all CD birds, AF^+ material in the zona externa of the anterior median eminence, the fiber layer of the median eminence, and the pars nervosa was small in amount or had completely disappeared. All CD birds had some degrees of diabetes insipidus; they drank much water, and the cage floors were always wet. However, the deafferentated area of the hypothalamus apparently maintained its normal structure as shown in Fig. 2; the tubero–eminential system was preserved during the experimental period.

Group 1. Operation Before Photostimulation

Complete deafferentation of the medial basal hypothalamus prevented photostimulated testicular growth (Table 1). CD birds had small, immature testes containing only spermatogonia and Sertoli cells. Control birds had developing testes containing spermatocytes. The cloacal protrusion of CD birds did not develop in contrast to the normal development observed in control birds (Fig. 3). In the adenohypophyses of the CD birds, PAS^+ basophil cells were small and infrequent as in immature birds. In the adenohypophyses of control birds, PAS^+ basophil cells were numerous and greater in volume in the central region of the cephalic lobe than was the case in the CD birds. Weight of the adenohypophyses of the CD birds was less than that of control birds (Table 1).

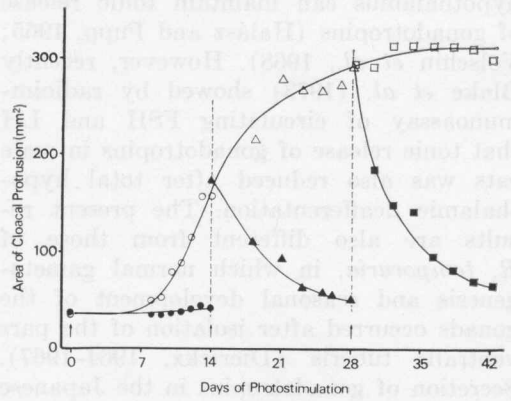

FIG. 3. Typical examples of development of cloacal protrusions of control (○, △, □) and CD (●, ▲, ■) quail. Arrows indicate day of operation.

In the birds receiving a FC, testicular growth was also prevented, whereas a RC did not prevent testicular growth (Table 1). In FC birds, one bird had somewhat larger testes (172 mg) than the others (35.6, 16.4, 8.79 mg). However, their cloacal protrusions were all small and undeveloped.

Adrenals and thyroids seemed active after complete deafferentation. Thyroidal weight of the CD birds was inclined to be less than that of control birds ($0.1 < P < 0.2$). However, the histological appearance of the thyroids in two groups was about the same.

Group 2. Operation After 2 Weeks' Photostimulation

Hypothalamic deafferentation was undertaken 2 wk after transfer to long daily photoperiods, and birds were kept under the same photoperiod for 2 more wk before autopsy. In the control birds the cloacal protrusion continued to develop and attained nearly the maximal level, but in the CD birds the cloacal protrusion decreased just after the operation and reached the initial level after 2 wk (Fig. 3). The testes also decreased to the initial state in the CD birds (Table 1); they showed only spermatogonia. In the control birds, the testes reached their highest levels; they contained spermatozoa. Adenohypophyses of the CD birds weighed less than those of the control birds (Table 1). Histologically, PAS^+ material seemed to accumulate in the basophil cells in the CD birds.

In both CD and control birds, adrenals and thyroids were similar in weight and histological profile.

Group 3. Operation after 4 Weeks' Photostimulation

Hypothalamic deafferentation was undertaken 4 wk after transfer to long days, and birds were kept 2 more wk on long days before autopsy. Testicular weight and the cloacal protrusion were their highest level in the control birds (Table 1 and Fig. 3). In the CD birds, the cloacal protrusion decreased just after deafferentation (Fig. 3), and the testes atrophied (Table 1). Histological features of the basophil cells

of the adenohypophysis in the CD bird were similar to those of the CD birds of Group 2; PAS^+ material seemed to accumulate in the basophil cells.

The thyroids of the CD bird were very low in weight compared to those of the control birds. However, the epithelial cells were rather high in this bird.

DISCUSSION

The present experiments indicate that deafferentation of the ventral hypothalamus inhibits photostimulated testicular growth. These findings may be correlated with the following results obtained mostly by other investigators.

(1) FRF and LRF are contained in the hypothalamus of the chicken (Kamiyoshi *et al.*, 1969; Tanaka *et al.*, 1969; Jackson and Nalbandov, 1969; Jackson, 1971a,b; Opel and Leopore, 1972). The basal hypothalamus of Japanese quail also contain LRF (Follett, 1970; Smith and Follett, 1972). Gonadotropin-releasing factor (GRF) seems to be synthesized in neurosecretory neurons in the nucleus tuberis of the hypothalamus as suggested by the fact that puromycin implanted into the nucleus tuberis inhibits photostimulated testicular growth (Wada, 1974). Lesion experiments also indicate that the basal infundibular nucleus (i.e., nucleus tuberis) is involved in photostimulated testicular growth and maintenance of gonadal activity in several species, including quail (Wilson, 1967; Graber *et al.*, 1967; Stetson, 1969, 1972a,b; Sharp and Follett, 1969; Ravona *et al.*, 1973a,b).

(2) The neurosecretory neurons in the nucleus tuberis might be regulated by both monoaminergic and cholinergic mechanisms. The neuropiles of the neurons are rich in the monoamine fluorescence in several species (Sharp and Follett, 1968; Oehmke, 1969; Oehmke *et al.*, 1969; Soest *et al.*, 1973) and in monoamine oxidase (Urano, 1968). Furthermore, electron microscopic observations showed that the neuropiles of the nucleus tuberis contain monoamine granules 100 nm in diameter (Oehmke *et al.*, 1969). Around perikarya of the neurons in the nucleus tuberis there are also axons containing synaptic vesicles in the house sparrow (Oehmke *et al.*, 1969). The nucleus tuberis of Japanese quail is innervated by axons that contain only synaptic vesicles (Wada, unpublished). Acetylcholinesterase is present in the perikarya of the nucleus tuberis of *Zosterops palpobrosa japonica* (Uemura, 1964). It seems likely, therefore, that the neurons producing GRF are controlled by both monoaminergic and cholinergic mechanisms.

Considering the findings mentioned above, it is plausible to conclude that, in the present experiments, axons that transfer photoperiodic information from the photoreceptor(s) to the nucleus tuberis were cut. This also indicates that the photoreceptor is located outside of the deafferentated area. After interruption of these axons the nucleus tuberis was not stimulated by light to release GRF and this resulted in atrophy of the testes.

After lesioning the dorsomedial nucleus in the hypothalamus (dorsal to the nucleus tuberis), testicular growth by photostimulation was abolished in Japanese quail (Sharp and Follett, 1969). The nucleus contains monoamine fluorescence (Sharp and Follett, 1968; Soest *et al.*, 1973). It is possible, therefore, that photoperiodic information is transferred to this nucleus at first and then relayed to the nucleus tuberis.

The results are different from those obtained in male rats, where the medial basal hypothalamus can maintain tonic release of gonadotropins (Halász and Pupp, 1965; Volschin *et al.*, 1968). However, recently Blake *et al.* (1973) showed by radioimmunoassay of circulating FSH and LH that tonic release of gonadotropins in male rats was also reduced after total hypothalamic deafferentation. The present results are also different from those of *R. temporaria*, in which normal gametogenesis and seasonal development of the gonads occurred after isolation of the pars ventralis tuberis (Dierickx, 1964–1967). Secretion of gonadotropins in the Japanese quail seems to be completely abolished after complete deafferentation of the medial basal hypothalamus. Variations

among species investigated may conceivably be due to differences in the degree of dependence upon the environmental photoperiodic change.

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