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Doris Phillips-Singh
Department of Zoology
Lucknow Christian College,
Golaganj, Lucknow-226018,
U.P., India.

Correspondence:
Doris Phillips-Singh
Department of Zoology
Lucknow Christian College,
Golaganj, Lucknow-226018,
U.P., India.

Interaction of circadian phase relation of neurotransmitters with L-thyroxine, alters differently, the gonadal activity of male and female weaver bird, *Ploceous phillipinus*

Doris Phillips-Singh

Abstract
The weaver bird is a photoperiodic sexually dimorphic species. The hyperthyroidic weaver birds administered with serotonergic and dopaminergic precursors at specific time intervals showed testicular quiescence in 8-h birds and full breeding conditions in 12-h and contrarily in females, 8-h induces ovarian development whereas 12-h leads to gonadal recrudescence. Thyroxine (T4) administration is gonadostimulatory in males and inhibitory in females. Combining the two treatments, the gonadostimulatory effect of T4 was superimposed by the inhibitory effects of 8-h relation in males while gonadostimulatory 8-h relation eliminated the inhibitory effects of T4 treatment in females. 12-h relation increased and decreased the degree of gonadal development in hyperthyroidic male and female birds respectively. Present findings demonstrate that this differential response may be due to exteroceptive and interoceptive factors related to puberty attainment.

Keywords: Male and female weaver birds, Neurotransmitters, Temporal synergism, Thyroid, Gonads.

Introduction:

In birds, the thyroid plays an important role in the growth and development of body and reproduction in both domestic [1, 2] as well as wild species [3, 4, 5, 6, 7, 8, 9]. The thyroid hormones also have a wide range of effects upon seasonal reproduction in birds [9, 10]. In male and female spotted munia, female weaver bird and male starlings thyroid ablation leads to unseasoned gonadal development and the abolition of the post-breeding gonadal regression [11]. Thyroidectomy also stops the development of photorefractoriness in birds and if carried out during the refractory period, leads to its spontaneous dissipation [2, 10, 12, 13, 14]. Recently it has been revealed that birds utilize thyroid not only for adaptive thermoregulation but, through the hypothalamo-hypophysial gonadal axis, the thyroids play important role in testicular regression in winter [15]. In the case of male weaver birds, thyroidectomy is reported to suppress gonadal development [16]. Thyroidectomy makes female weaver bird sensitive even to short photoperiod [17].

Neurotransmitters {(NTs) (serotonin and dopamine precursors)} on the other hand are reported to alter the reproductive as well as metabolic activities of several mammalian, reptilian, as well as avian species. In the case of birds both domestic i.e. Japanese quail [18, 19] as well as wild viz. Redheaded bunting [20], Spotted munia [21], Lal munia [22], Jungle bush quail, and rain quail [23] except in female Weaver bird [24] the 12-h relationship is gonadostimulatory and the 8-h relationship is gonadoinhibitory. To understand the action pathway of these drugs in birds several studies on the specific phase relationships of NTs and their interaction with photoperiod [25, 26] and several hormones [27, 28, 29] have been done. These studies suggest that the hypothalamo-hypophysial-gonadal axis may be the pathway for the action of these drugs.

Because of the above studies and the importance of the thyroid gland in the reproductive regulation of weaver birds, it was of interest and importance to study the interaction of the two NTs and the thyroids in both the sexes of the weaver bird.

Material and Methods

Weaver birds were collected from the vicinity of Lucknow (Lat. 26º 55' N, Long. 80º 59'E) during the progressive phase of the seasonal breeding cycle (May). Birds were fed with paddy (Oryza sativa). Both food and water were available ad libitum. The precursors of serotonin and dopamine were given to establish a specific phase relation of circadian serotonergic and dopaminergic activity [20] in the following manner –

The birds were divided into two main groups; A (Males), B (Female). Each group was further divided into six subgroups (N=6 in each) and were treated as follows-

Group I (Control): Normal Saline twice daily for 11 days

Group II (8-h+Veh): 5- Hydroxytryptophan (5-HTP, a serotonin precursor) at 8:00 A.M. and L-Dihydroxyphenylalanine (L-Dopa, a dopamine precursor) at 4:00 P.M. establishing an 8-h relationship between two injections and normal saline intraperitoneally (i.p.) at 6: 00 P.M.

Group III (12-h+Veh): 5-HTP at 8:00 A.M. and L-Dopa at 8:00 P.M. establishing a 12-h relationship) and normal saline (i.p.) at 10:00 P.M.

Group IV (T4): T4 at 8 A.M. and normal saline (i.p.) at 6:00 P.M.

Group V (T4+8-h): T4 and 5-HTP at 8:00 A.M. and L-Dopa at 4:00 P.M. establishing an 8-h relationship and T4 (i.p.) at 6:00 P.M.

Group VI (T4+12-h): T4 and 5-HTP at 8:00 A.M. and L-Dopa at 8:00 P.M. establishing a 12-h relationship and T4 (i.p.) at 10:00 P.M.

The 5-HTP and L-DOPA were given for 11 days [19, 20]. The birds were kept under very dim light (<1 lux) to avoid photoperiodic interference. The T4 (4µg per 100g body weight) was given for the whole study period. After 11 days all the groups were shifted to Normal Daylight (NDL). The testicular size (length and breadth) in males and the largest follicular diameter in females were measured initially, at Day 1 (1st day after treatment of 5-HTP and L-DOPA) and thereafter at Day 15, Day 30 and Day 45 (total period 11+45 = 56 days).

Statistical analysis

The data are represented as means ± SEM and were analyzed by one-way analysis of variance (ANOVA) followed by Dennett's test.

Results and Discussion

Results indicate that 5-HTP and L-DOPA (5mg/100g bodyweight for 11days under the constant condition of very dim light) given 8 hours apart {(8-h) (Group IIA)} leads to testicular quiescence (testicular volume, initial 56.57±0.62 final 32.62±0.60 mm3, P<0.001) while 12-h relation (Group IIIA) leads to a full breeding condition in male birds {(testicular volume, initial 57.17±0.35 and final 67.63±0.23 mm3, P<0.001) (Fig.1A)}. Contrarily in females, 8-h (Group IIB) induces ovarian development (initial largest follicular diameter 0.92±0.012 and final 1.34±0.03 mm, P<0.001) and 12-hr relation (Group IIIB) leads to gonadal inhibition (initial largest follicular diameter 0.92±0.02 and 0.54±0.02mm, P<0.001). On the other hand, T4 administration is gonadostimulatory in males {(Group IVA) (initial testicular volume, 56.02±0.25 and final 64.33±0.35 mm, P<0.001)} and inhibitory in females {(Group IVB) (initial follicular diameter 0.93±0.02 and final 0.70±0.02 mm, P<0.001)}. Combining the two treatments, it has been observed that gonadostimulatory effect of hyperthyroidism superimposed the inhibitory effects of 8-h relation in male weaver bird {(Group VA) (T4 final testicular volume 64.33±0.35)} and 8-h+T4 6.57±0.13 mm2 final, P<0.001 (Group VA) while gonadostimulatory 8-h relation eliminated the inhibitory effects of thyroxine treatment in females (Group IVb) {(T4, final follicular diameter, 0.70±0.02 mm and 8-h+T4, 1.22±0.01 mm final, P<0.001 (Group V b)}. On the other hand 12-h relation of 5-HTP and L-DOPA administration increased and decreased the degree of gonadal development in hyperthyroidic male {(Group VIA) (T4, Group IVA, testis volume final 64.33 ± 0.35 and T4+12hr (Group VIA final 83.46±0.52 mm2, P<0.001)} and hyperthyroidic female birds (follicular diameter, Group IVB, T4 final 0.70±0.02 mm and T4+12hr {(Group VIB) 0.42±0.01 mm final, P<0.001)} respectively (Figure1).

Results indicate that in male weaver birds the 8-h relationship seems to overpower the stimulatory effect of T4 and 12-h gives an additive push and exhibits significant reproductive growth and difference from the control birds {(P<0.001) (Fig.1A)} whereas 12-h shows opposite results. T4 administration (Group IVA) was gonadostimulatory in males like Group III (12-hr) birds. The T4+8h (Group VA) showed testicular development more or less similar to the saline-treated control group and the T4+12-h (Group VIA) showed advanced full breeding conditions (Figure 1A). Contrarily in female weaver birds, 8-h treatment induces ovarian development and 12-h treatment leads to gonadal inhibition. Interestingly, the T4 administration also led to gonadal inhibition contradicting the gonadal growth in the case of male birds. The T4+8-h and T4+12-h treatment showed more or less similar ovarian growth as the control birds which seems similar to the effect observed in the case of male birds after these two treatments i.e. T4+8-h and T4+12-h (Figure 1B).

These results observed in male and female weaver birds suggest the physiological significance of temporal synergism as the basis of seasonal reproductive conditions. The inhibitory or stimulatory effect of the two drugs given 8 or 12 hours apart replaces the stimulatory effect of T4 in males and the opposite in the case of females or vice-versa. This study gets support from the other studies, from Prof. Chaturvedi's group from Banaras Hindu University, Varanasi, India [18, 19, 20, 21, 22, 23,24,26, 29] suggesting that the present effect is due to specific phase relation of 5-HTP and L-DOPA converted into serotonin and dopamine only and not due to other biosynthetic products of these precursor drugs. Further, local activation of thyroid hormone within the hypothalamus may be the key factor in the regulation of seasonal reproduction [30]. It seems that thyroid is important in reproduction but its effect is overpowered by the stimulatory 12-h and 8-h treatments in the case of males and vice versa in the case of female weaver birds. It is therefore suggested that despite different environmental factors (photoperiod, humidity, etc.) used by different species to time their annual reproduction, the basic regulatory mechanism of seasonality appears to be the same.

The significant and opposite responses of male and female gonad to the specific phase relation of neurotransmitters as well as combined effects with thyroxine link the different responses of male and female birds with the exteroceptive

and interoceptive factors and also different physiological conditions regarding puberty attainment. The physiology of the two sexes seems to be different since hatching. The age of the mating partners is about six months in the case of males and about eighteen months in the case of females. Hence it seems that the absence of bright plumage in young and early first-year males keeps them safe from predators and therefore is of adaptive significance. These findings also suggest that the temporal phase relationship of neurotransmitters are involved in the differential reproductive regulations of the two sexes of this bird and interaction with thyroid strengthens the possibility for temporal synergism of neuroendocrine oscillations to be the basis of seasonality and also that the hypothamo-hypophysial-gonadal axis to be the pathway for the action of these NTs precursor drugs.

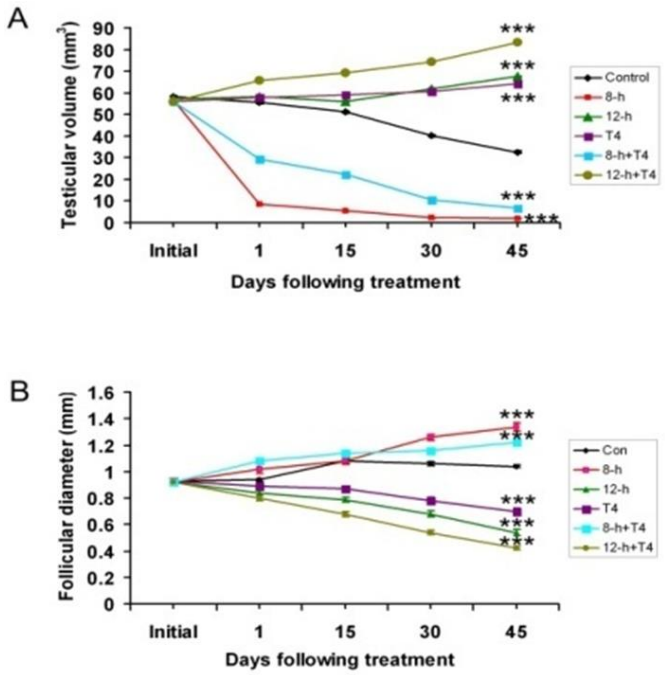

Fig.1: Testicular volume (A) and Follicular diameter (B) of Weaver birds administered 2 daily injections of normal saline (control); 5-HTP and L-DOPA at the interval of 8-h and 12-h for 11 days; and thyroxine (T4). Birds that received 11 days of injections of 5-HTP and L-DOPA at the interval of 8-h and 12-h received thyroxine (8-h+T4; 12-h+T4, respectively) injections for 56 days. Values are mean ± SE; the asterisk indicates the level of significant difference from the control group (***P<0.001).

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