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Temporal Phase Relation of Serotonergic and Dopaminergic Oscillations Alters Seasonal Reproduction and Adrenal Function in Jungle Bush Quail and Rain Quail

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Abstract: Present study investigates the role of temporal phase relation of serotonergic and dopaminergic oscillations in the regulation of seasonal reproduction in summer breeding Jungle bush quail, *Perdicula asiatica* and autumn breeding rain quail, *Coturnix coturnix coromandelica.* Two sets of experiments were performed in month of August, in reproductively quiescent (RQ) bush quail and reproductively active (RA) rain quail. In experiment I, both quails received the serotonin precursor, 5-HTP and the dopamine precursor, L-DOPA (5 mg/100 gm body weight/day for 13 days) at the interval of 8-hr or 12-hr. At the termination of the study (30 days post treatment), 8-hr suppressed and 12-hr increased body weight and testicular development compared to their respective controls in both the species of quail. However, in RA rain quail, testicular development in 12-hr group was similar to control. Further, in RQ bush quail, while adrenal activity of 8-hr group was more or less similar to control, 12-hr relation stimulated the activity of steroidogenic cords. On the other hand, in RA rain quail, control and 12-hr quail adrenal activity was similar while that of 8-hr showed suppression. In experiment II (quails receiving total dose of experiment I, i.e. 32.5 mg/100 gm body weight/ day for 2 days only, and sacrificed 30 days thereafter), response of the birds were similar to that of the experiment I. These findings indicate that, 8-hr and 12-hr relation of the 5-HTP and L-DOPA administration induced suppression and stimulation of the gonadal and adrenal function respectively, irrespective of the phase of the annual gonadal cycle of the two species of quail. It is concluded that specific relation of serotonergic and dopaminergic oscillation induces reproductively quiescent or active condition out of the season as a function of their time relation. These findings also suggest that, circadian organization may regulate reproductive and metabolic seasonality irrespective of the phase of the annual gonadal cycle.

Keywords: 5-Hydroxytryptophan (5-HTP), L-Dihydroxyphenylalanine (L-DOPA), testis, adrenal, Bush quail, Rain quail, seasonal reproduction.

1. INTRODUCTION

Reproduction in a majority of the birds occurs at a specific time of the year that is most favourable to the survival of the young and their parents. The environmental components used in timing the reproductive functions include photoperiod, rainfall, humidity, temperature and food in addition to other intrinsic mechanism(s) which may be hormonal or neural. In temperate zones, seasonal day length variation serves as the primary means of timing reproductive periodicity [1,2]. However, in most of the tropical and sub-tropical species environmental information (annual photoperiod) is not the primary regulator of annual gonadal cycle and initiation and termination of breeding varies according to the species. Studies on different species of birds have indicated the possibility of involvement of more than one environmental factor while direct influence of photoperiod in the process of regulation of annual reproductive cycle is also reported in some species similar to the photoperiodic species of temperate zones [3,4]. Although the proximate factor(s) in the tropics are not known, all the birds irrespective of their distribution and domestication respond to artificial illumination [5-12]. This indicates an ancestral capacity or the light may have a role in the stimulation or maintenance of the reproductive system even though it may not act as a synchronizer. Obviously most of the non-temperate zone birds if not all, although do not use daylength to time their seasonal reproduction but still respond to the artificial photoperiod.

In vertebrates, existence of circadian variation in endocrine secretions, hypothalamic factors and neurotransmitters is well reported [13]. The pattern of daily variation appears to change with physiological condition (viz. breeding, migration, molting, hibernation etc.), and with different seasons in seasonally breeding species. It is also reported that seasonal change in reproduction and other metabolic conditions (fattening etc.) may result from a temporal interaction of circadian neural oscillations that changes seasonally in their phase relations with one another. Meier and his group reported that daily phase relationship of two hormones i.e. corticosterone and prolactin may influence seasonal condition of migratory bird white throated sparrow, *Zonotrichia albicolis* [14]. This view was further supported when the effects of corticosterone and prolactin administered at different time intervals on a circadian basis could be duplicated by the administration of serotonergic and dopaminergic drugs given on the same ground [15-17]. Administration of these precursor drugs at 12 hour interval stimulated but at the 8 hour interval suppressed testicular function in many wild

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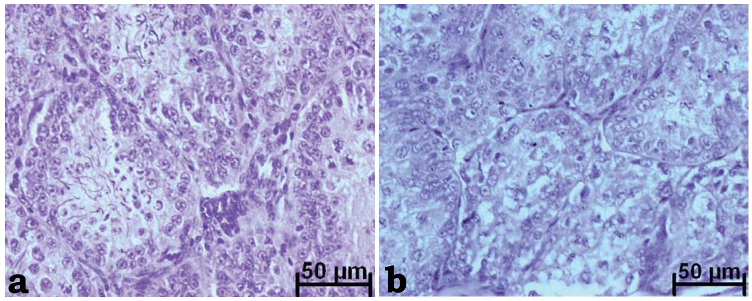

Fig. (5). Transverse section of testis of rain quail administered with 5-HTP and L-DOPA at 8 hour and 12 hour interval for 13 days (**a, b, c**) and 2 days (**d, e, f**). Compared to developed testis of control (**a** & **d**), 8-hr (**b** & **e**) quail testis shows atrophy, while degree of development is more in 12-hr (**c** & **f**) testis having bunches of spermatozoa. Scale bar = 50 µm.

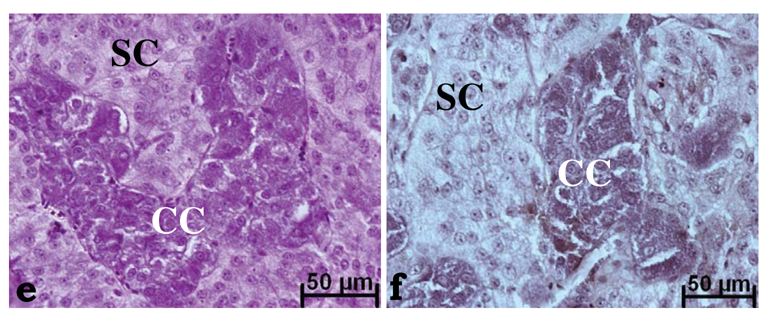

Fig. (6). Transverse section of adrenal of bush quail administered with 5-HTP and L-DOPA at 8 hour and 12 hour interval for 13 days (**a, b, c**) and 2 days (**d, e, f**). Compared to control (**a** & **d**) and 8-hr quail (**b** & **e**), cortico-medullary ratio has increased in 12-hr (**c** & **f**) quail along with hypertrophied cords. Scale bar = 50 µm.

ing condition with seminiferous tubules containing all the stages of spermatogenesis and spermatozoa. But, 8-hr (Fig. **5b**) rain quail testes showed suppressed condition with smaller seminiferous tubules and had no advanced stages of spermatogenesis except few layers of spermatogonial cells. However, testes of 12-hr (Fig. **5c**) rain quail showed full breeding condition as in control but appear to be more developed with enlarged seminiferous tubules containing increased number of spermatozoa in bunches and prominent Leydig cells in the interstitial spaces (Figs. **5a**, **b** & **c**). Transverse section of control rain quail adrenal (Fig. **7a**) showed active/hypertrophied condition. Prominent cords

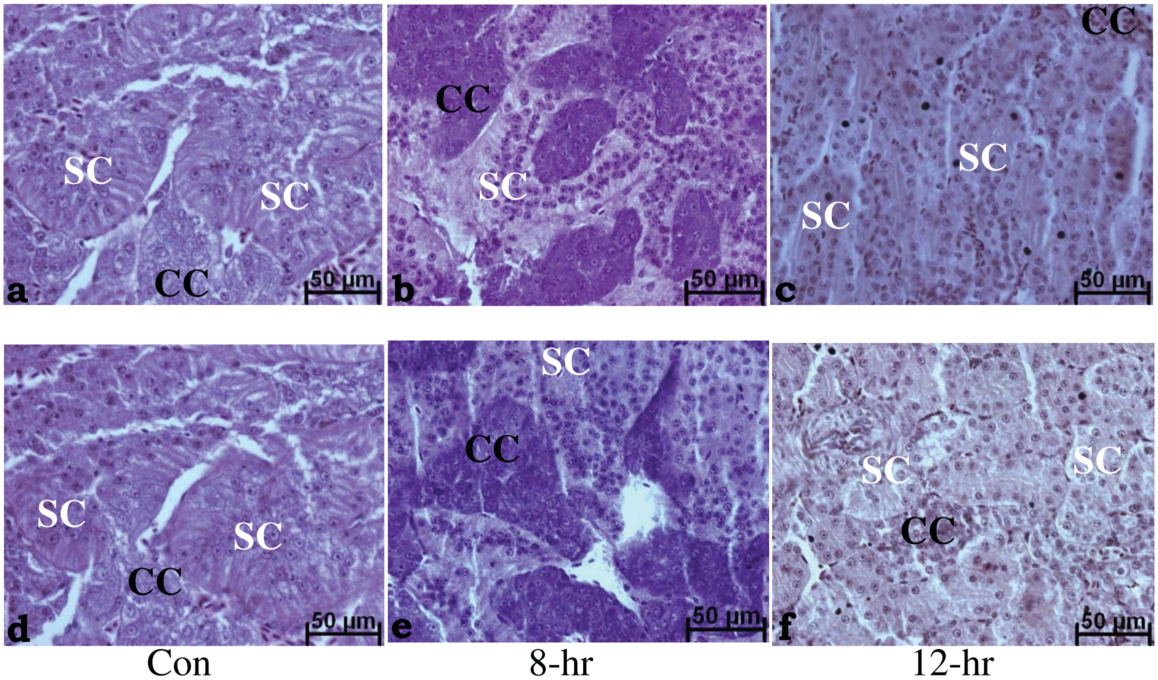

Fig. (7). Transverse section of adrenal of rain quail administered with 5-HTP and L-DOPA at 8 hour and 12 hour interval for 13 days (**a**, **b**, **c**) and 2 days (**d**, **e**, **f**). 8-hr (**b** & **e**) quail adrenal shows narrow steroidogenic cords (SC) and less cortico-medullary ratio compared to that of control (**a** & **d**). Although cords size is reduced in 12-hr quail (**c** & **f**) but overall surface area occupied by streoidogenic cords has increased and patches of medullary/ chromaffin cells (CC) are reduced significantly compared to control. Scale bar = 50 µm.

appeared as bigger mass due to folded condition and had clear nuclei and nucleoli. Adrenal of 8-hr quail (Fig. **7b**) had relatively narrow cords with somewhat smaller nuclei. In 12-hr quail (Fig. **7c**) as in control, highly vasculorized active adrenal had hypertrophied cords with prominent nuclei and nucleolus and relatively less number and area of patches of chromaffin cells. However, unlike folded cords of control, in 12-hr quail the cords were straight. In RA rain quail, cortico-medullary ratio was more in 12-hr and less in 8-hr compared to control (Fig. **8d**, **e** & **f**, Table **1**).

3.2. Experiment II

In experiment II, effects of 2 days of the injections of neurotransmitter precursor drugs on the body weight (Fig. **2B** & **2D**), testes weight (Fig. **3A** & **B**), testis (Fig. **4d**, **e** & **f** and Fig. **5d**, **e** & **f**) and adrenal (Fig. **6d**, **e** & **f** and Fig. **7d**, **e** & **f**) histology in both the species of quail were more or less similar to that of experiment I

4. DISCUSSION

Present study confirms that daily administration of serotonergic and dopaminergic precursors for either 13 days or 2 days, suppresses and stimulates gonadal development if given 8 hours or 12 hours apart respectively. It is interesting to note that both the species of the quail respond similarly to specific phase relation of serotonergic and dopaminergic oscillations irrespective of their annual gonadal condition [28,29]. 8-hr relation did not allow gonadal growth in bush quail similar to its control while 12-hr bush quail attained full breeding condition out of the season, which coincides with the peak reproductive condition of this species observed in the month of June. In rain quail also, 8-hr relation of the two precursor injections suppressed the gonadal development compared to the control which showed breeding condition, while the testes of 12-hr quail were in more advanced stage (Figs. **3-5**). Further this is the first report that indicates similar effectiveness of the total dose of the two precursor drugs, administered either in 13 or 2 days irrespective of the species and phase of their gonadal cycle.

It is obvious that present effect is not the pharmacological effect of serotonin and/or dopamine alone but is actually the effect of circadian phase relation of the two neurotransmitters on the neuroendocrine-gonadal axis which varies according to the interval between the two injections. Although we did not test in these species but in all our previous studies performed in males of different avian species including Japanese quail, out of six relations (0, 4, 8, 12, 16 and 20-hr), only 2 relations were found to be effective (8-hr gonado-inhibitory and 12-hr gonado-stimulatory) and rest of the relations were ineffective [22,25,26].

Although in the present study adrenal response also varied depending on the specific phase relation of serotonergic and dopaminergic oscillations / activities, it is yet to be tested if serotonin / dopamine alone can induce any effect on adrenal. However it is quite reasonable to suggest that it is the combined effect of the two drugs given at specific time interval hence producing the opposite effects in 8- and 12-hr quail adrenal. If this would have been the pharmacological or direct effect of serotonin and/or dopamine, response should be similar in both 8 and 12-hr birds. Reports are also there that serotonin induces glucocorticoid secretion from the adrenal cortex by stimulating the hypothalamo – hypophyseal axis and also by direct adrenocortical tissue stimulation [32, 33], although direct effect of dopamine on adrenal function is not known. Further, it is yet to be investigated

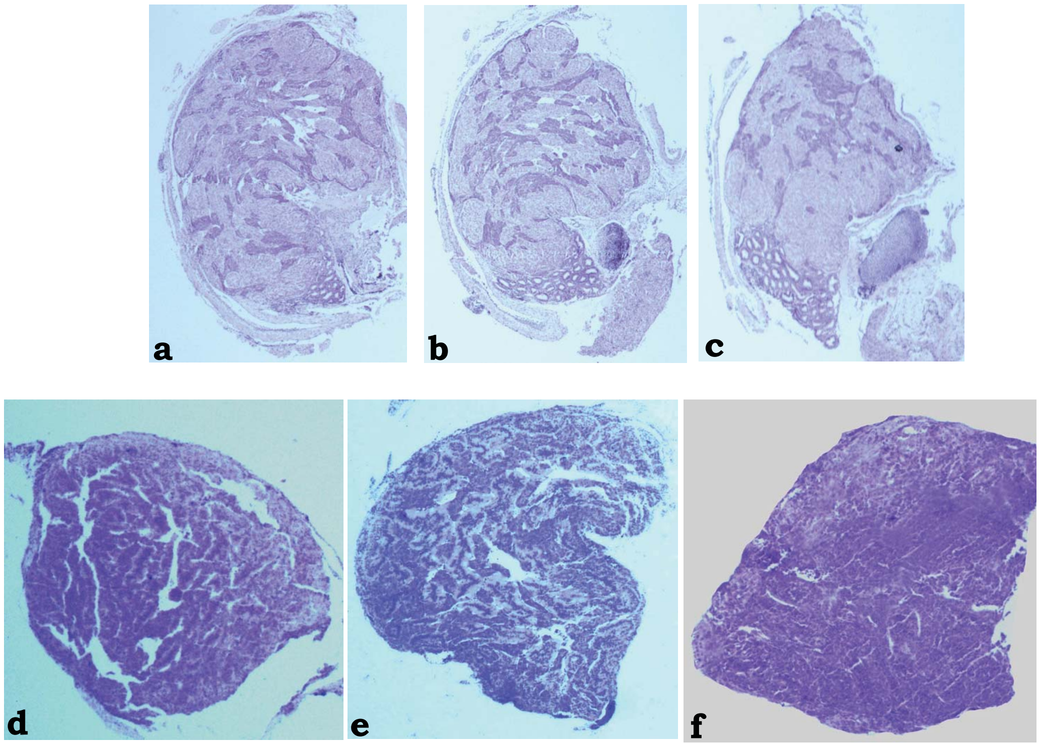

Fig. (8). Photomontage of adrenal of bush quail (**a**, **b** & **c**) and rain quail (**d, e** & **f**). Note increased amount of steroidogenic cords/corticomedullary ratio in 12-hr (**c**) Bush quail compared to control (**a**) and 8-hr (**b**) which are not significantly different from each other. In Rain quail, 8-hr (**e**) has decreased and 12-hr (**f**) adrenal has increased amount/area of steroidogenic cords and cortico-medullary ratio compared to control (**d**).

Table 1. Cortico-Medullary Ratio

Species	Treatment Period	Control	8-hr	12-hr
Bush Quail	3 Days	1.73:1	1.8:1	2.18:1
	2 Days	1.79:1	1.76:1	2.28:1
Rain Quail	13 Days	1.83:1	1.31:1	2.71:1
	2 Days	1.92:1	1.25:1	2.68:1

whether temporal synergism of serotonin and dopamine influences adrenal function directly or indirectly. Indirect evidence suggests that reproductive system by such treatments is influenced *via* higher brain center and/or altering hypothalamic/GnRH activity. But mechanism of temporal phase relation of serotonin and dopamine induced changes in metabolic responses (body weight and fat deposition) is yet to be explained. Since 8-hr is always gonado-inhibitory and 12- hr is gonado- stimulatory and body weight/ fat deposition if influenced, is suppressed and increased respectively, it is possible that adrenal activity / function runs parallel to metabolic/gonadal activity suggesting parallel gonad - body weight – adrenal relationship in these species.

It is interesting to note that not only the treatment of thirteen days but even two days of treatment could produce similar effect. This may be an interesting and applied way of reproductive regulation. It is worth mentioning that noticeable response occurs from 5th day onwards (2 days treatment + 3 days post-treatment) or on 5th day of ongoing 13 day treatment [34, 35]. Since, except 8- and 12-hr relation all other treatments were ineffective, it is reasonable to suggest that these are the effects of specific circadian phase/time relation between two drugs but not the individual/independent effect of either serotonergic or dopaminergic drug. It also can not be the combined effect of two drugs otherwise effects should have been similar in all the relationships. Most important point is that the two effective relations are having opposite effects.

Apart from gonadal response which is the major focus of the present as well as number of earlier studies, responses were seen in the body weight and fat deposition as well. In migratory bird red headed bunting, *Emberiza bruniceps*, which winters in India from Afghanistan and eastern Europe, enormous amount of migratory fat observed during spring migration may be abolished completely in 8-hr birds along with gonadal suppression, while 12-hr relation, may induce both fat deposition as well as gonadal stimulation out of the season [22]. Similar treatment when given in day old chicks of Japanese quail as well as three weeks old sexually immature quail, rate of growth (body weight gain) is significantly slow/less in 8-hr bird compared to control and 12-hr birds were significantly heavier. However, once attained the adult size/weight, there was no difference among the control and experimental groups, neither of the treatment induced any effect in the body weight response if given in adult Japanese quail [25].

This study also reveals that the adrenal cortical activity varies distinctly with the seasonal gonadal cycle of birds both in natural and experimental condition. Adrenal responses always maintained direct relationship with gonad in experimental conditions, confirming parallel gonad-adrenal relationship in these quail, under natural condition.

It is concluded that serotonergic and dopaminergic drugs when given 8 hours apart suppressed but when given 12 hours apart stimulated neuroendocrine-gonadal axis irrespective of the season, gonadal condition and the species. Further these effects appear to be the physiological effects since same drugs and doses produced different effects depending on the time interval of their administration. Moreover, not only the gonadal axis, but general metabolism (body weight) and adrenal function was also altered as a function of the time/ phase relation between the two circadian neural oscillations. These findings further strengthen the hypothesis that temporal synergism of serotonergic and dopaminergic oscillations may be the basis of seasonality.

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