Print ISSN: 0976-2876 Online ISSN: 2250-0138

Available online at: http://www.ijsr.in

INDIAN JOURNAL OF SCIENTIFIC RESEARCH

DOI:10.32606/IJSR.V13.I1.00006

Received: 13-05-2022 Accepted: 07-08-2022 Publication: 31-08-2022

Indian J.Sci.Res. 13 (1): 51-56, 2022 Original Research Article

ADMINISTRATION OF NEUROTRANSMITTER PRECURSOR DRUGS INFLUENCE NEUROENDOCRINE-GONADAL AXIS AND THEIR HORMONAL EXPRESSION IN DOMESTIC PIGEON, *Coloumba livia domestica*

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ABSTRACT

Current study was intended in an avian species which is monogamous i.e. *Columba livia domestica*. It produces crop milk during brooding. The effect of 0 hour and 8 hour relationships was tested on neuroendocrine-gonad interrelationship. Two neurotransmitter precursor drugs (5-HTP and L-DOPA) were used at different time intervals. Male birds were divided in to three groups, having eight individuals in each group. Group 1 as control received only vehicle. Group II was administered with 5-HTP and L-DOPA at 0 hr interval while Group III received 5-HTP and L-DOPA at 8 hr. Doses were 0.5mg/ kg body weight. Experimentations were terminated after 13 days and results were obtained 45 days post experimentation. Results indicate that daily injection of 5-HTP and L-DOPA, given at the interval of 0 hr and 8 hr, affected neurosecretory cells of the hypothalamic nuclei. Neurosecretory activity of the nuclei in form of the amount of Cresyl violet acetate stained neurosecretory material was seen to be moderate in saline treated control group and less in 0 hr birds but in 8 hr treated group of birds nuclei were heavily loaded with stained neurosecretory material. Hormonal estimations also revealed similar pattern of results i.e. 0 hr. group showed significantly decreased plasma LH (P<0.001) and testosterone (P<0.01) levels while 8 hr. group showed significantly increased plasma LH (P<0.001) and testosterone (P<0.001) levels in comparison to control. The present effect is a consequence of interaction (phase relation) of the two neural oscillations. Different temporal relationship of neurotransmitters apparently produced breeding and non-breeding condition respectively by 8 and 0 hr relationships. Hence it seems that seasonality is regulated by the phase relation of two circadian oscillators namely serotonergic and dopaminergic.

KEYWORDS: 5-HTP, L-DOPA, Neurosecretory cells, Domestic pigeon.

Seasonal breeding is a strategy which limits reproduction to specific durations of the year to maximize the chances of survival of the offspring. The adjacent environmental signals that control the timing of breeding season include changes in photoperiod, food availability and weather (Wingfield, 1983; Wingfield *et al* 1983; Wingfield and Farner, 1993). Birds breeding generally use changes in photoperiod as a long term analytical cue for the timing of breeding, with modifying cues supported by other environmental factors including temperature, food, rainfall and social interactions (Sharp, 1996; Wingfield, 1983).

It has been indisputably accepted that reproductive cycles result from various interacting external and internal factors. Of the several external factors light, temperature, rainfall, humidity, food availability and stimulus from mate are important in this regard. These ecological factors serve as synchronizer to stimulate higher brain centers and consequently neuroendocrine system for preparing the organism physiologically to reproduce in time. Among the internal factors, neuroendocrine-gonadal axis and hormones seem to play an important role in the regulation of reproductive cycles.

Current study was intended in such type of avian species which is monogamous and produces crop milk during brooding. The effect of hour relationships was tested on neuroendocrine-gonad interrelationship in domestic pigeon, *Columba livia domestica*.

MATERIALS AND METHODS

A colony of birds was maintained in the departmental aviary where they breed effectively. As per requirement adult pigeons had been chosen for study from the bulk. During the progressive phase of reproduction (December) 24 male pigeons were selected out by bilateral laparotomy from the group of acclimatized pigeons. They were divided into three groups of 8 birds each. Group I acted as the control group and received two daily injections of normal saline. Group II was 0 hr group in which birds received daily injections of 5-HTP (5-Hydroxytryptophan, a precursor of serotonin) and L-DOPA (L-Dihydroxyphenylalanine, a dopamine precursor) 0 hr apart i.e. at the same time at 6:00 AM. Group III was 8 hr group, where birds received

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daily injections of 5-HTP (5-Hydroxytryptophan, a precursor of serotonin, MW-220.23, cas 148290050, Product no. A0199225001 New Jersey USA 1-800 Across-01 Ceel, Belguim: +32.) at 6:00AM and L-DOPA (L-Dihydroxyphenylalanine, a dopamine precursor, MW-197.19 Batch no.0.1106 Product no.037079, Central Drug House (P) Ltd. Bombay.) 8 hr later i.e. at 2:00 PM. Drugs were administered at the dose of 5mg / 100 gm body weight in 1 ml normal saline for 13 days. After 45 days of termination of experiment, results were recorded.

Heparinized tubes were used for the collection of blood directly from left ventricle of the heart and plasma was separated and stored at -20 ^{o}C until assayed for plasma testosterone and LH concentrations by radioimmunoassay using commercial RIA kit, with the help of pathologist. Brain of both control and experimental pigeons were dissected out and fixed in bouin's fluid for at least 24 hours and processed for routine microtomy and histological observations. Sections was cut in 12µ thick paraffin section and stained with Cresyl violet acetate (pure high purity, MW-339.82, cas 10010-5, Product no. 929630050 New Jersey USA 1-800 Across-01 Ceel, Belguim: +32.) for the observation of hypothalamic nuclei according to the method described by Davenport (1960). The criteria for the assessment of neurosecretory activity were the quantity and nature of the neurosecretory materials (NSM) in various hypothalamic nuclei (Supra chiasmatic nuclei (SCN), Paraventricular nuclei (PVN), and Supra optic nuclei (SON)). The functional states of the neurosecretory activity in neurons of the SCN, PVN and SON have been evaluated in terms of the mean diameter of the cell nuclei and the quantity and quality of neurosecretory materials in the perikarya. Nuclear diameter was measured from five neurons from the different divisions of the SCN, PVN and SON each and from each group. The indices of the density of the NSM in the various nuclei were visually determined on an arbitrary scale of 0-6 as follows: none (0), light (1-2), moderate (3-4), normal (5), above normal (6). The index value of 6 was not found in control groups. Data was analyzed by Student's't' test, (Bruning and Kintz, 1977). Experiments were done in triplicates. The work was permitted by the departmental ethical committee. Birds were handled gently causing no cruelty.

RESULTS AND DISCUSSION

Results indicate that daily injection of 5-HTP and L-DOPA, given at the interval of 0 hr and 8 hr, affected neurosecretory cells of the hypothalamic nuclei. Neurosecretory activity of the nuclei in terms of the amount of neurosecretory material seen by Cresyl violet acetate staining was found to be moderate in saline treated control group and less in 0 hr birds. On the other hand in 8 hr later treated group of birds nuclei were heavily loaded (with neurosecretory material) neurosecretory cells. PVN of the saline treated control group showed moderate conditions. Cresyl violet stained neurons are closely packed with the perikarya. Variable quantity of NSM were found in NSC with moderate diameter of nuclei i.e. 23.24±1.50 µm [Table-1, Plate 1(row Ia)]. On the other hand birds treated with 5-HTP and L-DOPA at 0 hr interval showed poorly developed neurosecretory cells of PVN. Less quantity of NSM was loaded in neurosecretory cells and the diameter of neuronal nuclei was also found less i.e. 12.81±1.50 µm in comparison to control [Table-1, Plate 1(row Ib)]. In contrast the birds treated with 5-HTP followed by L-DOPA 8 hr apart showed extremely well developed condition. The neurons are distributed over a wide area. Neurosecretory material is seen in the form of large granules. The diameter was recorded 40.89±1.50µm which is significantly higher than control [Table-1, Plate 1(row Ic)]. The suprachiasmatic nuclei of saline treated control group were found in moderate condition. The diameter was 15.26±1.50 µm [Table-1, Plate 1(row IIa)].Treatment of 5-HTP and L-DOPA at 0 hr interval showed less active condition than control. The diameter was 12.00±1.26 µm which was found significantly lower than control [Table-1, Plate 1(row IIb)]. On the other hand treatment of these drugs at 8 hr interval showed well developed conditions of neuronal nuclei in SCN. The diameter was 38.47±0.98 µm [Table-1, Plate 1(row IIc)]. The supraoptic nuclei of the saline treated control group showed poorly developed but evenly distributed nuclei. Sometimes seen similar to the PVN but not as developed as PVN. The mean diameter of nuclei was found to be 18.46±0.98µm [Table-1, Plate 1(row IIIa)]. Birds treated with 5-HTP and L-DOPA at 0 hr interval showed inactiveness. Less quantity of NSM was found in NSC. The neuronal nuclei showed less diameter i.e. 11.20±0.80µm [Table-1, Plate 1(row IIIb)], while the 8 hr group showed enlarged nuclei with much quantity of NSM. The diameter was 30.42±0.98µm which was found much higher than control [Table-1, Plate 1(row IIIc)]. Hormonal estimations was also found similar pattern of results i.e. 0 hr. group showed significantly decreased plasma LH ($P<0.001$) and plasma testosterone ($P<0.001$) levels while 8 hr. group showed significantly increased plasma LH ($P<0.001$) and plasma testosterone ($P<0.001$) levels in comparison to control. (graph.1).

Table 1: Effect of 0 hr and 8 hr intervals relationship between 5-HTP and L-DOPA administration on the histochemical changes (Diameter of Neuronal nuclei) in the hypothalamic neurosecretory system of Domestic pigeon

Groups	Diameter of Neuronal Nuclei (µm) Mean ± S.E.		
	PVN	SON	SCN
Control	23.24 ± 1.50	18.46± 0.98	15.26 ± 1.50
0 hr	12.81 ± 1.50*$^{\downarrow}$	11.20± 0.80*$^{\downarrow}$	12.00 ± 1.26*$^{\downarrow}$
8 hr	40.89 ± 1.50**$^{\uparrow}$	30.42 ± 0.98**$^{\uparrow}$	38.47 ± 0.98***$^{\uparrow}$

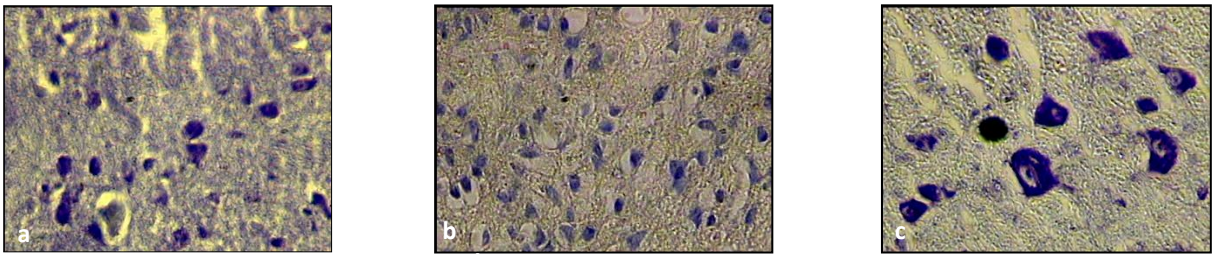
Significance of difference from control (*P<0.05, **P<0.01, ***P<0.001; Student's't'test)

Table 2: Effect of 0 hr and 8 hr intervals relationship between 5-HTP and L-DOPA administration on histochemical changes (Neurosecretory Index) in the hypothalamic neurosecretory system of Domestic pigeon on arbitrary scale

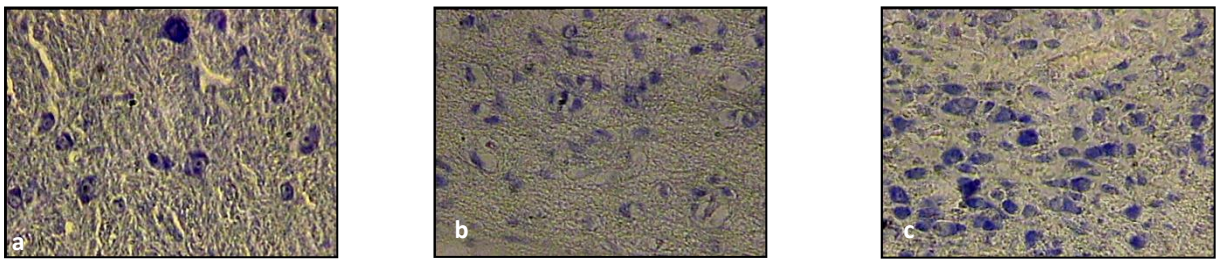
Groups	Neurosecretory Index		
	PVN	SON	SCN
Control	3-4	3-4	4
0 hr	1-2^{L}	1-2^{L}	2-3^{L}
8 hr	5-6^{H}	6^{H}	6^{H}

Keys: none (0), light (1-2), moderate (3-4), normal (5), above normal (6), L=low, H=high

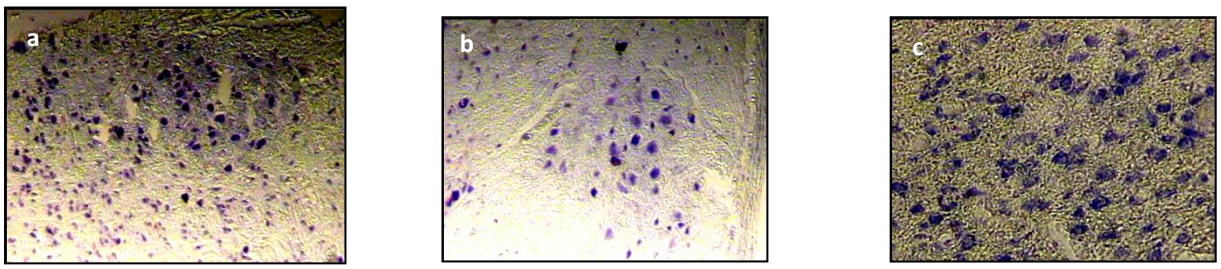
Plate 1: Photomicrographs of neurosecretory cells of hypothalamic nuclei stained with cresyl voilet acetate. X 40



Row I: Paraventricular Nuclie (PVN)



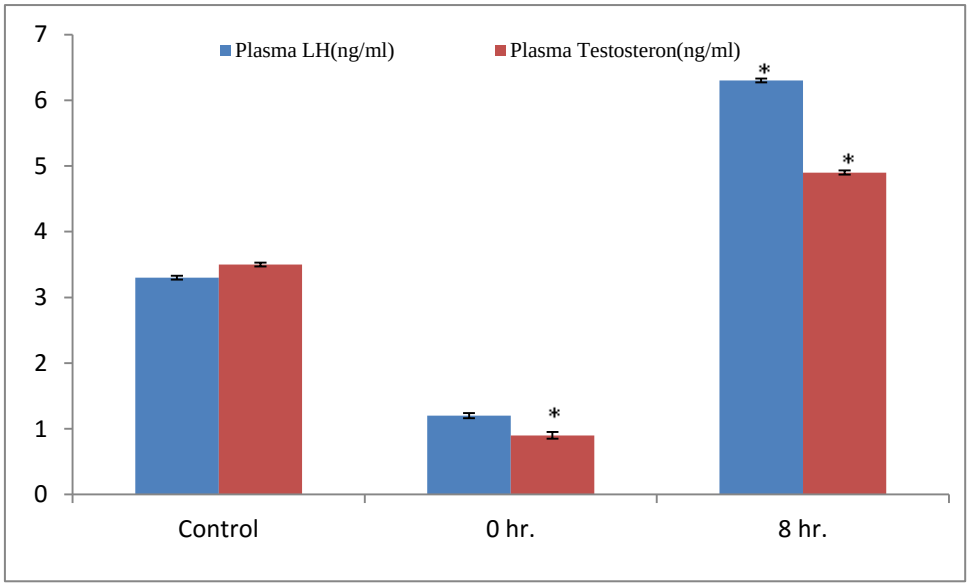
Row II: Suprachiasmatic Nuclie (SCN)



Row III: Supraoptic Nuclie (SON)

(Key: a, b and c in all rows represent group I Control, group II 0hr and group III 8hr respectively. Neurosecretory material is moderately seen in a (group I), poorly in b (group II) and heavily loaded in c (group III))

Graph 1: Effect of neurotransmitter affecting drugs on plasma LH and Plasma testosterone level of Domestic Pigeon



Values are Mean ± S. E.

Seasonality was first altered experimentally by daily injections of corticosterone and prolactin in the white throated sparrow and since the phase relations of the circadian rhythms of endogenous corticosterone and prolactin differ in photosensitive and photorefractory sparrows, it was proposed that the hormone rhythms are expressions of two circadian neuroendocrine oscillations that change seasonally in their phase relations and thereby regulate seasonality (Meier *et al*., 1971; 1981). Injections of corticosterone and prolactin are thought to set the phases of the two oscillations and many of their neural and hormonal circadian expression to produce seasonally appropriate relations and reproductive conditions. It was further proposed that the hormones reset the seasonal conditions by way of their known effects on the brain neurotransmitters, serotonin and dopamine; although, the role of other neurotransmitters cannot be ruled out. A temporal interaction of circadian neuroendocrine rhythms may well prove to be the primary organizational basis for reproductive regulation in view of central nervous system regulation of gonadal development in birds (Kuenzel, 2000).

As in the birds in general (Gabe, 1966) the system contains more or less scattered groups of NSCs. Especially stricking is the well developed PVN. The SON is rather poorly developed. Kripalani (1967) also reported that PVN is more prominently developed that of SON in eight species of birds (one passerine and seven non-passerine) investigated by her. The hypothalamo-hypophysial neurosecretory system of birds has been extensively studied by several investigators (Benoit and Assenmacher, 1959; Kobayashi *et al*., 1961; George and Naik, 1965; Bern *et al*., 1966; John and George, 1967; Singh, 1972; Singh and Dominic, 1974, 1979). Some studies on the effect of photoperiodic changes on the neurosecretory activity in birds have also been carried out (Oksche *et al*., 1959; Oksche, 1960; Bern *et al*., 1966; Farner and Oksche, 1962). George and Naik (1965) have studied the hypothalamo-hyphophysial system in a migratory starling, *Sturnus roseus*, with special reference to the cyclic changes occurring during the pre- and post-migratory periods. These authors observed that the release of the neurosecretory material from the hypothalamus a few days prior to migration may be considered as a trigger for migration. In the light of these observations John and George (1967) further studied the neurosecretory system of some other species of migratory birds with special reference to their migratory activity.

Present findings suggest that daily injections of 5-HTP and L-DOPA given at the difference of 0-hr and 8-hr apart actually affected the neuroendocrine-gonadal axis and their hormonal expression (i.e. LH and Testosterone). Possibly these treatments of only thirteen days reset the switch of neuroendocrine mechanism resulting in gonado-suppressive or stimulatory response. Therefore, it is suggested that in nature too, there exists specific phase relationship between circadian neural oscillation which change seasonally and may be the basis of specific breeding or non-breeding condition. This assumption also gets indirect support from following earlier reports.

(a) Circadian variation has been reported in brain / hypothalamic neurotransmitters (serotonin and dopamine) which are reported to have significant neuroendocrine function affecting reproduction. Further, circadian changes of these neurotransmitters are also reported to be influenced by photoregimn/light:dark condition and hormones (Wilson and Meier, 1989; Mai *et al*., 1994; Pan, 1996; Shieh *et al*., 1997).

(b) Circadian peak of neurotransmitter activities occur at different time in different breeding conditions (Morin *et al*., 1977; Khan and Joy, 1988; Tiwari *et al*., 2006).

Different responses (8-hr stimulatory and 0 hr inhibitory) of neuronal nuclei and hormonal levels as a function of time relation between the serotonergic and dopaminergic activity (similar to breeding and non-breeding condition of annual cycle) suggests that these treatments did not produce any pharmacological effect of drugs on body metabolism but altered the seasonality of *Columba livia* through metabolic/physiological alterations. Obviously the present effect is the consequence of interaction (phase relation) of the two neural oscillations. Different temporal relationship of neurotransmitters apparently produced breeding and non-breeding condition respectively by 8 and 0 hr relationships. This study supports the hypothesis that seasonality is regulated by the phase relation of two circadian oscillators namely serotonergic and dopaminergic.

ACKNOWLEDGEMENT

Financial support from UGC Government of India for the first author in the form of Post Doctoral fellowship for women is gratefully acknowledged. Departmental facilities are also acknowledged.

REFERENCES

Benoi J. and Assenmacher I., 1959. The control by visible radiations of the gonadotropic activity of the duck hypophysis. Recent Progr. Hormone Res., **15:** 143-164.

Bern H.A., Nishioka R.S., Mewaldt L.R. and Farner D.S., 1966. Photoperiodic and osmotic influences on the ultrastructure of the hypothalamic neurosecretory system of the White-crowned sparrow, Zonotrichia leucophrys gambeli., Z. zellforsch. Mikroskop. Anat. **69:** 198-227.

Bruning J.L. and Kintz B.L., 1977. Computational Hand Book of Statistics, II ed., Scott, Foresman, California, Glenview I. Ltd. Pp. 8-10.

Davenport H.A., 1960. Histological and Histochemical techniques. W.B. Saunders.

Farner D.S. and Oksche A., 1962. Neurosecretion in birds. Gen. Comp. Endocrinol., **2:** 113-147.

Gabe M., 1966. Neurosecretion. Pergamon Press, Oxford.

George J.C. and Naik D.V., 1965. The hypothalamo-hypophysial neurosecretory system of the migratory starling, sturnus roseus (Linnaeus). J. Anim. Morphol. Phyiol., **12:** 42-56.

John T.M. and George J.C, 1967. Cyclic histochemical changes in the hypothalamo-hypophysial neurosecretory system of the migratory wagtails, motacilla alba and motacilla flava. J. Anim. Morphol. and Physiol., **14:** 216-222.

Khan I.A. and Joy K.P., 1988: Seasonal and daily variation in hypothalamic monoamine levels and monoamine oxidase activity in the Teleost Channa punctatus (Bloch). Chronobiol. Intern., **5(4):** 311 – 316.

Kobayashi H., Bern H.A., Nishioka R.S. and Hyodo Y., 1961. The hypothalamo-hypophyseal neurosecretory system of the parakeet, Melopsittacus undulatus. Gen. Comp. Endocrinol., **1:** 545-564.

Kripalani K., 1967. Micro-anatomic studies on the avian hypothalamo-hypophyseal system in eight indian species. Proc. Zool. Soc., Calcutta, **20:** 103-113.

Kuenzel W.J., 2000. Central nervous system regulation of development in the avian male. Poul. Sci., **79:** 1679-1688.

Mai M.L., Shieh K.R. and Pan J.T., 1994: Circadian changes of serum prolactin levels and tuberoinfundibular dopaminergic neuron activity in ovariectomized rats treated with or without estrogen: The role of the suprachiasmatic nuclei. Endocrinology, **60:** 520-526.

Meier A.H., Martin D.D. and Mac Gregor R. III, 1971. Temporal synergism of corticosterone and prolactin controlling gonadal growth in sparrows. Science, **173:** 1240-1242.

Meier A.H., Ferrell B.R. and Miller L.J., 1981. Circadian components of the circannual mechanism in the white-throated sparrow. In: Proceedings of XVII, International Ornithological congress. Deutsche, Ornithology-Gesellschoft. pp. 458-462.

Morin L.P., Fitzgerald K.M., Ruska B. and Zucker I., 1977. Circadian organisation and neural

mediation of hamster reproductive rhythms. Psychoneuroendocrinology, **2:** 73-98.

Oksche A., 1960. Optico-vegetative regulatory mechanisms of the diencephalon. Anat. Anz., **108:** 320-329.

Oksche A., Laws D.F., Kamemoto F.I. and Farner D.S., 1959. The hypothalamo-hypophysial neurosecretory system of the White-crowned sparrow, Zonotrichia leucophrys gambeli., Z. Zellforsch. Mikroskop. Anat., **51:** 1-42.

Pan J.T., 1996. Neuroendocrine functions of dopamine. In: CNS Neurotransmitter and Neuromodulators: Dopamine. Stone, T.W. ed., pp 213-232, Boca Raton, CRS Press.

Sharp P.J., 1996. Strategies in avian breeding cycles. Anim. Reprod. Sci., **42:** 505-513.

Sheih K.R., Chu Y.S. and Pan J.T., 1997. Circadian change of dopaminergic neuron activity: effects of constant light and melatonin. Neuroendocrinology, (Neuroreport), **8:** 2283-2287.

Singh K., 1972. Studies on the hypothalamo-hypophysial complex in birds. Ph.D. Thesis, B.H.U. Varanasi, India.

Singh K. and Dominic C.J., 1974. Failure of chloral hydrate to block the hypertonic saline-induced changes in the hypothalamic neurosecr- etory system of the Spotted owlet, Athene brama Temminck. Indian J. Expt. Biol., **12:** 490-494.

Singh K. and Dominic C.J., 1979. Hydrocortisone inhibition of hypertonic saline-induced changes in the hypothalamic neurosecretory system of the Spotted owlet, Athene brama Temminck. Indian J. Expt. Biol., **17:** 1053-1057.

Tiwari A.C., Kumar P., Singh S., Sharma D. and Chaturvedi C.M., 2006. Reproductive phase dependent circadian variation in hypothalamic concentration of serotonin, dopamine and peripheral thyroxine level in Japanese quail following 5-HTP and L-DOPA administration at specific time intervals. Biol. Rhythm Res., **37 (1)**: 73-86.

Wilson J.M. and Meier A.H., 1989. Resetting the annual cycle with timed daily injections of 5-hydroxytryptophan and L-dihydroxyphenylananine in Syrian hamsters. Chronobiol. Intern., **6:** 113-132.

Wingfield J.C., 1983. Environmental and endocrine control of avian reproduction: An ecological approach. In: Avian reproduction, S. Mikami, K. Homma, and M. Wada eds: Environmental and ecological perspectives. pp. 265-288, Springer Verlag, Berlin.

Wingfield J.C. and Farner D.S., 1993. Endocrinology of reproduction in wild birds. In: Avian Endocrinology. D.S. Farner, J.R. King and K.C. Parkes eds. pp. 263-327, Academic Press, London.

Wingfield J.C., Moore M.L. and Farner D.S., 1983. Endocrine response to inclement weather in naturally breeding populations of white crowned sparrows, Zonotrichia leucophrys pugentensis. Auk., **100:** 52-62.