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SHORT COMMUNICATION

# PHYSIOLOGICAL EVIDENCE OF AN INVERSE CORRELATION BETWEEN THE GONADAL FUNCTION AND RFRP-3 NEURONS IN MICE

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**Abstract:** The temporal phase relation of circadian neural oscillations (serotonergic and dopaminergic) are reported to account for seasonal physiological conditions including reproduction. The circadian rhythms of hormones (corticosterone and prolactin) appear to be important expression of these oscillations and by feed-back mechanism hormonal rhythms may not only maintain the neural oscillations (serotonergic and dopaminergic) but may also entrain each other. Further, in this direction and on the assumption/evidence that hormonal rhythms are the expression of neural rhythms, 5-hydroxytryptophan (5-HTP), a rate limiting precursor substrate for serotonin, was substituted for corticosterone, and L-dihydroxyphenyalanine (L-DOPA), a rate-limiting precursor for dopamine, was substituted for prolactin. Although so far most of the studies have concentrated on the serotonergic and dopaminergic oscillations possibly due to their central effect, role of other oscillations cannot be ruled out. Our study presented in this communication deals with the temporal phase relation between two neural oscillations (serotonergic and dopaminergic) that are conveyed to regulate seasonal reproductive and other metabolic/physiological conditions. Our work tested the applicability of this mechanism in continuous breeder Parkes (P) strain mice during postnatal gonadal development and attainment of puberty with respect to the expression of a hypothalamic gonadotropin-inhibitory peptide, RFamide-related peptide-3 (RFRP-3). Further, age-dependent variations were also monitored in the expression of *ir*-RFRP-3 in mice brain and the testicular activity to assess the possible correlation between the gonadal function (development, maturation and regression).

**Key words**: 5-Hydroxytryptophan (5-HTP); L-dihydroxyphenylalanine (L-DOPA); RFamide-related peptide-3 (RFRP-3), Reproduction, Temporal Synergism.

## INTRODUCTION

Reproduction involves precisely timed neural and endocrine events which occur within a well-defined schedule and seem to be synchronized with external and internal factors such as photoperiod, availability of food and favorable conditions, neural inputs and hormonal balance etc. (Kennaway *et al.*, 2012). Neural, endocrine, biochemical and behavioral rhythms provide the basis for temporal organization of reproductive functions which also involve temporal synchronization of reproductive events within the population as well as between the individual and the external environment. Effects of all the external and/or internal factors regulating reproduction are funneled through hypothalamo-hypophyseal-gonadal axis. However, gap/site between various stimuli and stimulation of hypothalamic neurons involved directly in reproduction was not yet defined.

In general, much attention has been paid to investigate the regulation of seasonal reproduction in vertebrate species but it was not yet clear whether recurrent gonadal activation observed in each breeding season and its regulation was physiologically similar to the gonadal activation observed first time during puberty attainment or not. Since, stimulation of reproductive system during both the events are funneled through hypothalamo-pituitary-gonadal axis it was thought worthwhile to investigate the factor (s)/ mechanism (s) at the level of higher brain center which may modulate gonadal axis. Although enormous literature was focused on the physiology of reproduction, relatively less attention was paid on the regulatory mechanism which initiates this process.

Several reports have hypothesized and experimentally proved that temporal phase relation of circadian neural oscillations may induce specific physiological (reproduction)/ metabolic conditions (Jaiwal and Chaturvedi, 2013; Yadav and Chaturvedi, 2014; 2015) and hence may be the basis of seasonality but its role in the continuous breeders was not investigated. This short communication deals with the temporal phase relation between two neural oscillations (serotonergic and dopaminergic) that are reported to determine seasonal reproductive and other metabolic/ physiological conditions. This commentary also tests the applicability of this mechanism in continuous breeder Parkes (P) strain mice during postnatal gonadal development and attainment of

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puberty with respect to the expression of a novel hypothalamic gonadotropin-inhibitory peptide RFamide-related peptide-3 (RFRP-3).

Keeping regulation of reproduction in mind, this short communication was designed to answer the following questions:

1. What is the role of circadian organization/temporal synergism of circadian serotonergic and dopaminergic oscillations in the regulation of gonadal development in mice during puberty attainment and in the modulation of RFRP-3 expression in brain?
2. What is the physiological role of RFRP-3 in the reproductive regulation of developing (prepuberal) mice and is there any age-dependent variation in the activity of gonad and RFRP-3 neurons?

Experiments were designed to study the effect of temporal synergism of neurotransmitters (serotonin and dopamine) on the testicular development of mice and the reproductive aspects of RFamide-related peptide-3 (RFRP-3), a mammalian ortholog of avian GnIH in mice. Specific phase relation of serotonergic and dopaminergic oscillations was induced by the administration of their precursors 5-HTP (5-hydroxytryptophan) and L-DOPA (L-dihydroxyphenylalanine) respectively. These drugs were injected daily (5mg/100g body weight) at different intervals over a period of 13 days. Results indicated that daily injections of serotonergic and dopaminergic precursor drugs (5-HTP & L-DOPA) given at different time intervals induced variable effects on the reproductive development and age-dependent body weight gain in mice. Out of the six relationships, 8-h relation between the administration of serotonergic and dopaminergic drugs leads to the suppression of gonadal growth and related changes in the spermatogenesis, plasma testosterone level, sperm count, motility and viability while increased activity was observed in 12-h mice compared to the control (Sethi and Chaturvedi, 2009).

To test the internal coincidence model with respect to testicular development in mice, seven experimental groups of two-week-old mice were administered with 5-HTP daily at 8:00 A.M., while L-DOPA was administered at different time intervals in different groups establishing 6, 7, 8, 9, 10, 11 and 12-h relationship between the two injections. Both 5-HTP and L-DOPA were administered intraperitoneally at a dose of 5mg/100g body weight for 13 days under $LL_{dim}$ and then shifted to a 12-h photoperiod (LD 12:12) until the termination of the experiment, i.e. 11 days post-treatment. Results of this study indicated that the gonado-suppressive effect starts from 7-h group attaining maximum reproductive inhibition in 8-h mice. These findings clearly indicated that temporal phase relation of neural oscillations may influence reproductive development in prepubertal mice and suggest the influence of circadian organization in the development of neuroendocrine-gonadal axis (Sethi and Chaturvedi, 2010).

To investigate the functional aspects of RFRP-3, a mammalian ortholog of avian GnIH during different reproductive conditions, prepuberal mice (3 weeks old) were divided into 3 groups. Group 1 served as control and received two injections of normal saline daily, while other 2 experimental groups were administered with 5-HTP daily at 8:00 A.M., and L-DOPA was administered at different time intervals in both the groups i.e. at 4:00p.m. and 08:00p.m. for 13 days establishing 8- and 12-h phase relations, respectively between the two injection of the precursor drugs. Results indicated that an increased expression of RFRP-3 in the dorsomedial nuclei of hypothalamus of prepubertal mice decreased significantly in the postpubertal condition. Administration of 5-HTP and L-DOPA at specific time interval not only altered the testicular activity but also induced simultaneous changes in the RFRP-3 neurons of DMH (an increase in 8-h and a decrease in 12-h mice). The present study provided the evidences of inverse correlation between RFRP-3 neurons and gonadal function of mice during control as well as experimental conditions (Sethi *et al.,* 2010a).

To study age-dependent variation in the expression of hypothalamic RFRP-3 and its correlation with gonadal maturation and function, age-dependent variations were also monitored in day-old, 1, 3, 5, 7, 9, 11, 13-week and 1.5 year-old mice with the expression of *ir*-RFRP-3 in mice brain and the testicular activity to assess the possible correlation between the gonadal function. Our findings demonstrated decreasing or decreased expression of *ir*-RFRP-3 in the brain of developing mice with increasing or increased plasma testosterone level (Sethi *et al.,* 2010b).

The overall findings indicated that administration of 5-HTP and L-DOPA at specific time (8 or 12 h) intervals not only altered the testicular activity but also induced simultaneous changes in the expression of RFRP-3 neurons of DMH nuclei (Sethi and Chaturvedi, 2015, communicated). The 8-h phase relation exhibited degenerative changes in the seminiferous tubules of the testis with an increased activity of RFRP-3 neurons while in the 12-h mice, complete spermatogenic complement in the seminiferous tubules indicated full breeding condition with decreased activity of *ir*-RFRP-3 neurons in the

DMH. Thus, in addition to the classical stimulatory role of GnRH in the control of pituitary-gonadal axis, RFRP-3 is also involved as an inhibitory peptide in the reproductive regulation.

Based on these findings, it is concluded that 8-h relation between the administration of 5-HTP and L-DOPA (as in seasonally breeding species studied so far) leads to anti-spermatogenic effects in mice and that the effects are reversible because it does not induce complete testicular atrophy but only suppresses the testicular development. Further, the present work opens up a new avenue of research to support the role of circadian system in testicular development during the maturation of gonadal axis even in the continuous breeder. In addition, this study also provides an experimental paradigm to test the coincidence model. These findings provide first physiological evidence of an inverse correlation between the gonadal function and RFRP-3 neurons supporting GnIH-like activity of this neuropeptide in mice.

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