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Interactive effect of light colours and temporal synergism of circadian neural oscillations in reproductive regulation of Japanese quail

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ABSTRACT

Avian literature reports the modulation of 'photoperiodic gonadal responses' by the temporal phase relation of serotonergic and dopaminergic oscillations in Japanese quail. But, the modulation of 'light colour responses' by the temporal synergism of neural oscillations is not yet known. Hence the present study was designed to investigate the interaction of the light colour (blue, red) and the phase relation of neural oscillations in the reproductive regulation of Japanese quail. Three week old male Japanese quail were divided into two groups and maintained under a long day length condition (16 L:8D) and were exposed to a 30 lux intensity of blue LED (light emitting diode) (B LED) and a red LED light (R LED). At the age of 15.5 weeks, quail of one subgroup of B LED were injected with serotonin precursor (5-HTP) and dopamine precursor (L-DOPA) 12 hrs apart (B LED + 12-hr) and those of the R LED group were injected with the same drugs (5 mg/100 g body weight over a period of thirteen days) but 8 hrs apart (R LED + 8-hr). The remaining subgroups of both the light colour groups (B LED & R LED) received normal saline twice daily and served as controls. Cloacal gland volume was recorded weekly until 35.5 weeks of age when the study was terminated and reproductive parameters (testicular volume, GSI, seminiferous tubule diameter and plasma testosterone) were assessed.

Results indicate that the 8-hr temporal phase relation of neural oscillations suppresses reproductive activity even during the photosensitive phase of the red light exposed quail (R LED + 8-hr) compare to the R LED controls. On the other hand, the 12-hr temporal phase relation stimulates the gonadal development of the B LED + 12-hr quail compared to the B LED controls which after completing one cycle entered into a regressive phase and remained sexually quiescent. These experiments suggest that the temporal phase relations of circadian neural oscillations, in addition to modulating the classical photoperiodic responses, may also modulate the gonadal responses to blue (suppressive) and red (stimulatory) light. These studies led us to conclude that the temporal phase relation of serotonergic and dopaminergic oscillations is not only an important regulator of avian reproduction but may also override the classical effects of light colours in Japanese quail.

1. Introduction

An enormous literature is focused on the effect of the most important and reliable environmental cue, the photoperiod or day length, on the gonadal responses of birds including Japanese quail [11,12,16,17,22,30,32,35]. Studies have focused on the underlying mechanism of the photo-sexual responses [26,43,46] but reports on the effects of different light colours and intensity on reproductive performance are limited and the underlying mechanism of such effects is not known precisely (quail- [21,57]; hen- [25]; chick- [28]; black headed bunting- [31,33,34,36–38,44]).

In a photoperiodic species, all the three variables of light, the duration (day length/L:D cycle), quantity (intensity- lux) and quality (colour wavelength) might affect the photo-sexual responses. However, these variables may be synergistic to each other as suggested by various reports. For example, at the same intensity, short wavelength light (blue or green) is interpreted as an inhibitory photoperiod suppressing the gonadal growth as compared to light of long wavelength (red) which results in a greater magnitude of gonadal response [1,31,34,44,45]. Further, the same duration of photoperiod (L: D cycle) may act as a longer photoperiod (day length) at high light intensity (brighter) as compare to low light intensity (dim) [2,7,8]. A recent report on Japanese quail clearly indicates these synergistic photoperiodic effects of different light colours and intensity on long day quail [57]. Accordingly, low intensity (30 lx- LED) short wavelength light (blue) induced gonadal suppression even under long days (LD 16:8) exhibiting a condition similar to absolute photorefractoriness, unlike control (the high intensity, 100 lx white fluorescent light) which maintained quail gonads continuously in the active photosensitive phase. Interestingly, low intensity (30 lx LED) but long wavelength light (red) also maintained gonad continuously in a developed condition similar to 100 lx white light control [57].

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In addition to exogenous factors, a number of reports also suggest the role of specific phase relations of serotonergic and dopaminergic oscillations in the reproductive regulation of many seasonally breeding avian species including Japanese quail (see [14]). According to this regulatory factor it is logical that temporal phase relations of different circadian hormonal or neural oscillations vary seasonally because circadian activities of different hormones/neurotransmitters do vary with different seasons and physiological conditions [29,49,53,54]. Hence experimentally it was possible to simulate specific seasonal breeding condition by duplicating the specific phase relation of two neural/hormonal oscillations [14,39–41,52,53,55]. Based on this assumption, six different temporal phase relation of serotonergic and dopaminergic oscillations were induced by administering the serotonin precursor 5-hydroxytryptophan (5-HTP) and dopamine precursor L-dihydroxyphenylalanine (L-DOPA) at intervals of 0, 4, 8, 12, 16 and 20 hrs establishing 0-h, 4-h, 8-h, 12-h, 16-h and 20-h phase relation between the two neural oscillations. Since serotonin and dopamine do not cross the blood brain barrier but their precursors (5-HTP and L-DOPA respectively) do cross and are converted to neurotransmitters (serotonin and dopamine respectively) by appropriate neurons of the brain, systemic injections of 5-HTP and L-DOPA were given instead of serotonin and dopamine injections respectively [48,51]. This protocol resulted in the suppression of gonadal growth in 8-h and stimulated reproductive performance in 12-h quail, while other time relations were found to be ineffective [9,13]. This mechanism appears to be comparable with the internal coincidence model of reproductive regulation [55].

Further, in this series of studies it was interesting to note that 8-h phase relation of the two oscillations was capable of masking the effect of long photoperiod and the effect of a short photoperiod may be abolished by injections of 5-HTP and L-DOPA if given 12 h apart [3,5,6,10,13,56]. These interactive studies of photoperiod/day length duration and specific phase relations of neural oscillations suggest that latter may modulate the photoperiodic effects of birds by simulating long (gonadostimulatory) and short day (gonado-inhibitory) effects [14,56]. However, unlike Japanese quail, where relative photorefractoriness can be dissipated by 5-HTP and L-DOPA administration if given 12 h apart (12-h relation); in the migratory Red headed bunting (*Emberiza bruniceps*), the 12-h relation of these injections was unable to terminate absolute photorefractoriness during the post-reproductive phase of the annual gonadal cycle [4], However, the same treatment during quiescent and progressive phase of the annual gonadal cycle, could stimulate gonadal development significantly as compared to control [4].

In view of the fact that light intensity and wavelength also influence gonadal growth, it was thought worthwhile to investigate if the specific temporal phase relations of neural oscillations may also modulate the effects of light quantity (intensity) and quality (colour) on the reproductive development of Japanese quail. To test this hypothesis, quail maintained under blue light (having inhibitory effect on reproduction) were administered 5-HTP and L-DOPA at the interval of 12 h (having gonado-stimulatory effects). Contrarily, quail maintained under red light (having a stimulatory effect on reproduction) were administered with serotonin and dopamine precursors at the interval of 8 h (having a gonado-inhibitory effect).

Thus the specific aim of the present study was to compare the relative potency of exogenous factors i.e. light colours and intensity and an endogenous factor the temporal phase relation of serotonergic and dopaminergic oscillations on the reproductive regulation of Japanese quail.

2. Materials and Methods

Three week old male Japanese quail purchased from Chuck Gazaria Farm, Sultanpur Road, Lucknow, were divided into 2 groups ($n = 10$ in each) and exposed to 30 lx intensity of Blue LED (B LED) or Red LED (R LED) light under long days (LD 16:8) in separate photoperiodic wooden chambers containing LED bulb of blue and red light respectively. These bulbs were connected to a time switch (timer) set for 16 h light and 8 h dark condition ('on time' was at 0600 and 'off time' was at 2200 daily). Food was commercial chicken ration (Finisher grower) purchased from Santosh poultry feed centre, Maldahiya, Varanasi, India. Both food and drinking water was provided ad libitum. The experiment was conducted in accordance with institutional practice and within the framework of the revised Animals (Scientific Procedures) Act of 2002 of the Government of India. Quail were monitored weekly for their cloacal gland volume, a reliable external indicator of androgenic activity [47,50]. Twelve weeks after the exposure of light colours, i.e. at the age of 15.5 weeks, both the groups were divided into two subgroups ($n = 5$). One subgroup of blue light (B LED) and red light (R LED) quail received normal saline (0.9%) twice daily for a period of 13 days and served as blue/red light controls. Another subgroup of B LED quail were administered with the serotonergic drug (5-HTP) at 0800 am and the dopaminergic drug (L-DOPA) at 2000 i.e. at the time interval of 12 h (B LED + 12-h quail). Likewise, another subgroup of R LED quail were treated with 5-HTP (at 0800) and L-DOPA (1600 pm) at the interval of 8 h (R LED + 8-h) over a period of 13 days.

The cloacal gland volume of quail of all the groups was recorded weekly during the post-treatment period up to 35.5 weeks of age. At the terminations of study i.e. 35.5 weeks of age (or 18.5 weeks post treatment), the lengths and widths of the cloacal glands were again measured in situ by dial calipers and blood was collected, from the wing vein into a heparinized tubes and centrifuged at 4000 rpm for 20 min at 4 °C. Plasma was separated and stored at −20° for hormonal assays to be performed later. For the present study, plasma testosterone levels were measured using an EIA kit (DSI s.r.l., Italy) according to the manufacturer's protocol. The antiserum used in the assay was 100% specific for testosterone (cross reactivity/specificity with testosterone was 100%); the cross reactivity of the assay was 0.056% with progesterone, 0.004% with cortisol, 0.005% with estradiol, 4.8% with dihydrotestosterone, 3.6% with androstenedione, 0.048% with androsterone, 0.004% with cortisone, 0.002% with estriol and 0.007% with estrone. The analytical sensitivity of the assay was 0.0576 ng/ml. The intra-assay coefficient of variation (CV) was 5.6% whereas the inter-assay CV was 7.1%. Accuracy for this assay was 99%.

Thereafter, quail from each group were weighed, anaesthesized with thiopentone and dissected. The length and width of the left testis was measured in situ with dial calipers. Both the testes were excised and weighed to calculate the GSI (Gonadosomatic index) and thereafter testes were fixed in Zamboni's solution for histological studies. The GSI was calculated as the weight of the paired testes/100 g body weight. The cloacal gland and testicular volumes were calculated using Bissonett's formula $4/3\pi ab^2$ (a = half of the long axis; b = half of the short axis) [12,27]. For routine histology, after 24 h of fixation, the testes were transferred to an ascending series of alcohol for dehydration, cleared in xylene followed by paraffinization. Six micron thick sections of paraffin embedded tissue were cut by a Weswox rotary microtome (Western Electric and Scientific Works, Ambala Cantt, India) and processed for routine histology using hematoxylin and eosin stain. The stained histological sections were viewed under a microscope (Axioskop 2 Plus; Carl Zeiss AG, Oberkochen, Germany) and images were captured with a digital camera. The diameter of the 10 seminiferous tubules/section was measured in 10 sections from each testis using an occulometer and micrometer.

All the numerical data [testicular volume (cm^3), GSI (gram testes weight per 100 g body weight), seminiferous tubule diameter (μm) and plasma testosterone concentration (ng/ml)] were analyzed by One-way ANOVA for the comparison of group means followed by post hoc test Dunnett T_3 and significance was assumed at the level of $p < 0.05$. Weekly data (no. of weeks 1–33 = 3.5–35.5 week age) of cloacal gland volume (cm^3) in different light colours (1 = blue and 2 = red LED light) with different type of treatments (5-HTP and L-DOPA at specific time intervals, i.e. 8 h and 12 h apart, establishing 8-h and 12-h