Response of skeleton photoperiod in male brahminy myna (*Sturnus pagodarum*)

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Abstract

The present study was suggested that in two pulse light:dark (LD) cycle paradigm (two light pulse at fixed intervals in a 24 hr LD cycle), that constitutes a "skeleton photoperiod", the Ist light pulse longer in duration entrain CRPP and IInd light pulse usually shorter falling in the night (Φi) induces photoperiodic responses. Considered together, it has been proved that a skeleton photoperiod becomes effective as a single complete photoperiod. Acclimatized birds were exposed to 6L:5D:1L:12D, 6L:9D:1L:8D, 6L:13D:1L:4D and 8L:16D for 35 days after this all all four groups were transferred to long photoperiod (15L:9D). In this experiment observations were made on body mass and testis size at the beginning, end and also on appropriate intervals during the experiment. Testis induction was faster in group IV after 35 days in comperision to other three groups. Observations that occurred in groups that received different light pulses of variable time period during which course of illumination of Φi was different. The photoperiodic reactions are regulated by the endogenous circadian rhythm of photoperiodic photosensitivity (CRPP).

Key words: Male brahminy myna, testis, body mass, skeleton photoperiod or night interruption experiment

INTRODUCTION

Photoperiodism regulates processes of the seasonality of many biological processes because change in annual pattern of day length is regular everywhere except at the equator. Night-interruption experiments test the validity of the external coincidence model in avian photoperiodism. These experiments, also called the Skeleton photoperiods or Scotophase scan experiments, are based on the concept as outlined in the Bünning-Pittendrigh model that shows the effects of a light pulse would vary depending where it falls in a 24 h day. Thus, such an LD cycle consists of a short light period (6-8 h) coupled with dark period (18-16 h), in which is usually given a short light pulse of 0.25 to 2.0 hours once per cycle at temporally fixed points. Thus, two light periods tend to simulate the effects of corresponding long light period. Jenner and Engels (1952) were first to use night interruption experiments in the avian photoperiodism. Using *Zonotrichia albicollis*, they showed that a 2 h light given at 14 or 16 h after light on of a 6 h main short photoperiod induced gonadal growth in the photosensitive white-throated sparrows, but the same 2 h light given at other time points failed to cause photoinduction. Later, these experiments were applied on a number of species including *Passer domesticus* (Murton *et al*., 1970*); Coturnix coturnix japonica* (Wada, 1981); *Chloris*

chloris (Murton *et al*., 1970a); *Passer montanus* (Lofts and Lam, 1973); *Ploceus philippinus* (Singh and Chandola, 1981 and Rani et al., 2007); *Emberiza melanocephala* (Tewary and Kumar, 1984), *Sturnus pagodarum* (Kumar and Kumar, and 1993) and *Meleagris gallopavo* (Siopes and Proudman, 2008). In this protocol, the first light period (called the entraining light pulse, E-pulse) given early in the subjective day 'entrains' the CRPP, and so the photoinducible phase (Φi) begins some 'fixed' hours later in day. Thus, the E-pulse entrains photoperiodic response curve and decides the timing of the Φi. Light coinciding with the Φi initiates photoperiodic reaction. Therefore, the strength (duration, light intensity, or light wavelength) of the E-pulse could influence the photoperiodic induction. About three and half decades ago, Follett and Sharp (1969) provided first evidence that in Japanese quail exposed to daily skeleton photoperiods, a decrease and an increase in the duration of E-pulse advances and delays, respectively, the Φi. Singh *et al*. (2002) also found the effects of duration and the timing of the E-pulse on photoperiodic induction in the black headed bunting. In birds, both retinal and extra retinal structures are involved in the photoperiodic photoreception. The extra retinal photoreceptors (ERP's) are localized in pineal and hypothalamus (Infundibular complex and Para olfactory lobe). Whereas in the pineal individual cells (pinealocytes) are photo receptive, in hypothalamus photic information is relayed by deep brain photoreceptor (DBP) to reproductive axis, photoperiodic time measurement (PTM) is effected by 'Clock' which in birds appears to be multiunit. At least three units of the Avian Clock, the retina, the pineal and the hypothalamus, have been identified and it has been proposed that these three work in negative feedback loop model (Cassone and Menaker, 1984). Whether avian photoperiodism is independent of the interactions of clock components or whether, it is regulated by clock (s) away from these three identified clock structures remains to be investigated. It is intriguing that photoperiodism in birds appears to be based on the interaction of light with the endogenous circadian oscillator. The photoperiodic reactions are regulated by the endogenous circadian rhythm of photoperiodic photosensitivity (CRPP). Notably, birds were the first vertebrates in which evidence for CRPP underlying PTM was demonstrated (Hamner, 1963).

MATERIAL AND METHODS

Male birds were acclimatized to captive conditions under natural day lengths for two weeks before they were exposed to experimental conditions. Once in every month, birds received glucose and vitamins and antibiotic (Tetracycline hydrochloride). Birds were divided in four groups (n=6 each) and kept in cage (size $45\times25\times25cm^3$). Birds were fed ad libitum on mixture of seeds of *Pennisetum* and *Setaria*. Indoors, day-night situation was provided by artificial illumination, by switching 'on' and switching 'off' of the fluorescent tubes or CFL lamps (Phillips) providing cold white light at an intensity of 500 at perch level light. Automatic time switches (Muller clock) controlled the light and dark periods. The experiment started on August 2005. Birds were grouped in four groups (n=6 each). These groups were exposed to 6L:5D:1L:12D (gp-1), 6L:9D:1L:8D (gp-2), 6L:13D:1L:4D (gp-3) and 8L:16D (gp-4). Observations on body mass and testis volume were taken in the beginning and at monthly intervals. Body mass was recorded on a top pan balance providing an accuracy of 0.1 gm. The testicular size was assessed by laparotomy under local anaesthesia (Kumar et al., 2001). The dimensions of the left testis were recorded, and testis

volume was calculated by from 4/3 лab^2, where л = 22/7, where a and b denote half of the long and short axis, respectively. The data from the experiment was analyzed by one-way analysis of variance (one-way RM ANOVA). Newman-Keuls Multiple Comparison Tests compared different means if ANOVA indicated the significance of difference between mean values. Significance was taken at $P<0.05$.

RESULTS

The result of this experiment shows that there was slight gain in mean body mass in all groups with the increase in the duration of treatment under different photoperiods. The mean body mass among the four groups had significant difference but magnitude was different among all groups [1-way RM ANOVA: group1, $F_{3, 9}=27.78$, $P< 0.0001$; group2, $F_{3,9}=11.28$, $P=0.0021$; group3, $F_{3,9}=5.817$, $P=0.0108$; group4, $F_{3,9}=5.210$, $P=0.0233$]. There was no testicular growth in birds exposed to skeleton and short photoperiods. Testis were regressed by the day 35. After 35 days when the group were transferred to long days (15L:9D) there was a marginal growth of testis in the group III (6L:13D:1L:4D) but the condition was not maintained without any further growth on 60th day of Long day exposure. In the other two skeleton groups (group I and II) there was no increase in the testis size. Birds were exposed to8L:16D (group IV) on re-exposure to 15L:9D after 35 days showed a significant ($P<0.05$) testicular growth and futher growth was found by 60th day of long day exposure. The mean testicular volume among the four groups had significant difference among all groups [1-way RM ANOVA: group1, $F_{3,9}=25.26$, $P=0.0001$; group2, $F_{3,9}=6.219$, $P= 0.0142$; group3, $F_{3,9}=6.090$, $P=0.0151$; group4, $F_{3,9}=34.97$, $P =0.0001$].

DISCUSSION

The traditional view of how skeleton photoperiods can stimulate long days is based upon concept of how circadian rhythms might be used as a day length-measuring device. It is assumed that a rhythm of 'photosensitivity' exists somewhere within the brain. Normally, this coincidence occurs only at one season of the year when the day lengths are of sufficient length to engage the photosensitive phase, but certain skeleton photoperiods can mimic this situation, one of the light pulses coinciding with the period of peak photosensitivity. It is suggested that in a two-pulse light: dark (LD) cycle paradigm (two light pulse at fixed intervals in a 24 hr LD cycle), that constitutes a 'skeleton' photoperiod (SKP), the first (usually longer) light pulse (main photoperiod) entrain CRPP and the second (usually shorter) light pulse falling in the night (ϕi) induces photoperiodic responses. Considered together, this means that a SKP will be as effective as a single complete photoperiod (CP, a single continuous light period in a 24 hr LD cycle) in influencing the photo-neuroendocrine system in a photoperiodic species.

There was no testicular growth in birds exposed to skeleton and short photoperiods. Testis were regressed by the day 35. After 35 days when the group were transferred to long days (15L:9D) there was a marginal growth of testis in the group III (6L:13D:1L:4D) but the condition was not maintained without any further growth on 60th day of Long day exposure. In the other two skeleton groups (group I and II) there was no increase in the testis size. Birds were exposed to8L:16D (group IV) on re-exposure to 15L:9D after 35 days showed a

significant (P<0.05) testicular growth and futher growth was found by 60th day of long day exposure. When considered together with the observations that occurred in the groups that received different light pulses in which course of illumination of ϕi was different, it seems that there is a differential sensitivity in the course of ϕi to light. This is logical given that the photoperiodic machinery is regulated by the circadian system (Kumar and Follett, 1993), and that there can be a time course for magnitude of the response to light in components of the circadian timing system (e.g., suprachiamatic nuclei, SCN, intereniculate leaflet, IGL, retina etc.; Teclemariam-Mesbah *et al*., 1995).

However the claim of time-dependent photoinductiveness needs to be further confirmed using rapid markers of photoperiodic responses (e.g., rise in LH following single day photostimulation) since the testis growth response is the summation of the inductive effects of light pulses over a number of days (Lofts, 1975) during which the differential effects of LD cycles, if any, may have been compromised. A major problem in studying the light effects on the CRPP is that it cannot be monitored directly. Therefore, formal analyses of the CRPP are done by looking at parallel circadian rhythms (other 'hands of the clock', e.g., activity rhythms). There was a difference in the rate and magnitude of response between the complete and skeleton photoperiods. It appears that the subtropical house sparrow uses photoperiodic strategy in regulation of its seasonal testicular responses similar to that is reported for its temperate population (Anushi and Bhardwaj, 2006). The phase response curve (PRC) for the circadian activity rhythm does not tell exactly what happens to the photoperiodic oscillator, as the pacemakers (s) for photoperiodic time measurement (PTM) could be different from those governing other circadian rhythms, viz. feeding, drinking, locomotion or melatonin rhythms (Juss *et al*., 1995). In conclusion illumination of a larger course of ϕi results in higher rates of gonadal growth but there is a limit above which there will be no further increase in the testis growth and, maybe, in the secretion of gonadotropins hormones. It is clear that different portions of ϕi have different sensitivity to light, i.e., there is a phase response curve (PRC) for light.

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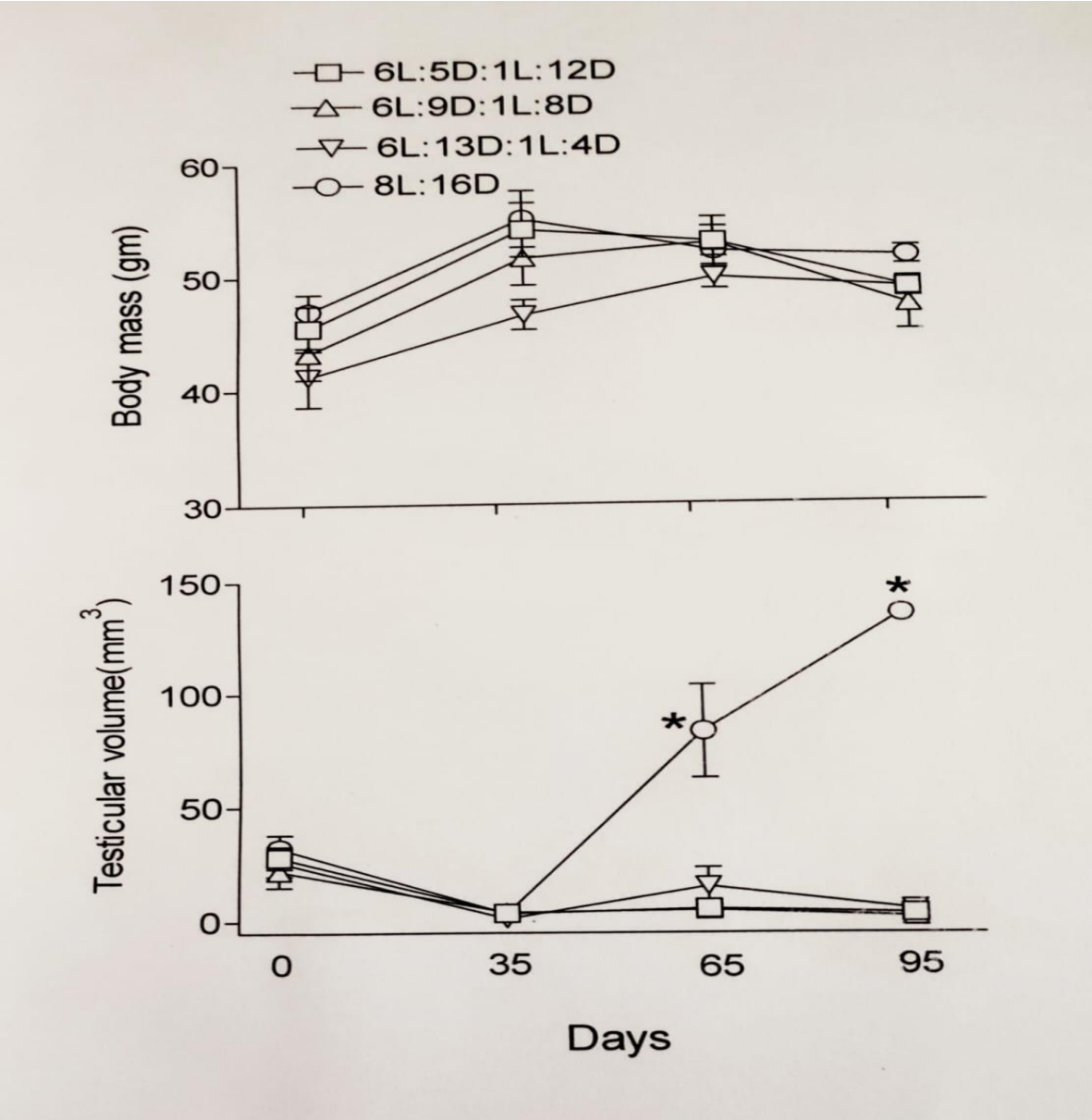

Figure 1a: Values are means ± S.E.M. shows the change in body mass under different skeleton photoperiods and short photoperiod. Birds were transferred to Long photoperiods (15L:9D) after 35 days.

Figure 1b: Values are means ± S.E.M. shows the change in testicular volume has been measured under different skeleton and short photoperiods. Birds were transferred to 15L:9D after 35 days.