

Effects of light intensity on circadian activity behaviour in the Indian weaverbird (*Ploceus philippinus*)

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Circadian (locomotor activity/perching) behaviour of the weaverbird (*Ploceus philippinus*) under different light intensities was studied. Six groups of birds were subjected to 12L:12D (L = 1000 and 10 lux and D = 0.3 lux) for two weeks, and thereafter released into constant dim illumination (LL_{dim} = 0.3 lux). After two weeks of LL_{dim}, birds were given a 2 h light pulse of 1000 lux at circadian time (CT) 12, 17 and 20, and exposure of LL_{dim} was continued for another two weeks and the activity pattern was monitored. As expected, all birds were entrained under 12L:12D showing dense-activity in the group that was placed under light phase of 1000 lux. Under LL_{dim} birds exhibited circadian activity rhythms with periods longer or shorter than 24 h. Light pulse at CT 12 caused small delay shift in the activity phase, but a larger delay in phase shift occurred when the pulse was given at CT 17. A pulse at CT 20 caused small advanced phase shift. Thus, photoperiodic weaverbird appears to show circadian system regulated behaviour as seen by activity-rest pattern under programmed light cycles.

Keywords: Circadian period, Circadian rhythm, Light intensity, Light pulse, Phase shift

Light is the major environmental factor for entrainment of the circadian activity rhythms¹. The daily light-dark cycle is the reliable zeitgeber for entraining the endogenous circadian rhythms in an organism. Different environmental stimuli such as duration, intensity and spectral composition of light, temperature, availability of food and social interaction etc. have shown to synchronize these endogenous circadian rhythms of organisms. Entrainment of endogenous circadian periodicity depends upon the period of the light-dark cycle, the photic response characteristics of the species (as depicted in phase-response curves), and on a number of other factors that are not yet fully understood^{2,3}, but circadian system has the ability to integrate zeitgeber information in a phase dependent manner⁴ that also affect the entrainment⁵. In an earlier study it was shown that birds have the ability to entrain to light-dark cycle of different light intensities during light period but they free-run under constant dim light and became arrhythmic under constant bright light⁶.

Under natural condition, the diurnal animals are active during day-time as can be confirmed under simulated laboratory condition. They entrain to light-

dark condition, and free-run under constant illumination (LL_{bright} or LL_{dim}) or darkness (DD), with a circadian period (τ) longer or shorter than 24 h. Studies demonstrating the phase shifts caused by various factors such as light, temperature and drugs, have contributed to the understanding of circadian clock⁷. The phase shift response of light pulses during subjective night is the fundamental property of circadian clock. The strength of the zeitgeber affects the magnitude of the ensuing shifts for example, larger phase shifts are attained by the light pulses that are longer or brighter⁸ or of certain wavelengths^{9,10}. The timing of light pulses can delay or advance circadian rhythms in birds¹¹ as well as in mammals¹². Certain studies have reported single pulses of bright light at two different phases of circadian rhythm, demonstrating phase-dependent phase-shifting¹³. The magnitude of phase-shift by light is dependent upon the intensity and duration of the light¹³. In most studies, the light pulses introduced at late subjective night caused phase advances, and that during daytime produced little or no phase shift¹⁴.

In weaver birds (*Ploceus philippinus*) the locomotor activity is affected by different day and nighttime light intensities¹⁵, therefore, It has been aimed to investigate whether dim and bright light intensities would affect the amplitude of perception and synchronization of circadian activity behaviour to

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the programmed light-dark cycles. Also, an attempt has been made to demonstrate if, the light pulses given during early, mid and late subjective night would affect the circadian characteristics of locomotor activity under constant conditions.

Materials and Methods

Experiments were performed on photosensitive male Indian weaver birds. Weaver birds were captured using mist nets in Meerut ($29^{\circ} 01' N$; $77^{\circ} 45' E$), India and kept in an outdoor aviary ($3 \times 2.5 \times 2.5$ m) for one week before being moved to photoperiodic chambers where they received artificial light: dark (LD) cycle. Six groups of birds of 5 birds each were subjected to 12L:12D for two weeks with bright (1000 lux: groups 1, 2 and 3) and dim (10 lux: groups 4, 5 and 6) day time light intensities. Also, all groups received 0.3 lux light intensity at night time. After 2 weeks under 12L:12D, they were transferred to constant dim light (LL_{dim}) for further two weeks. Thereafter, the birds were given 2 h light pulses (1000 lux) at CT 12 (groups 1 and 4), CT 17 (group 2 and 5) and CT 20 (group 3 and 6) (circadian time, CT 0 = time of activity onset) only for one day and then they continued being under LL_{dim} .

The birds under experiment were housed singly in activity cages (size = $61 \times 31 \times 40$ cm) kept inside the photoperiodic boxes (size = $74 \times 56 \times 72$ cm). The locomotor activity was considered as a reliable assay for the study of circadian property and was easily measured¹⁶. The Chronobiology Kit software from Stanford Software Systems, Stanford, California, USA, was used for recording and analysis of the locomotor activity of bird¹⁶. The recording of locomotor activity (actogram) was double-plotted. Changes in circadian period were observed for selected 10 days under LD and LL_{dim} by the Chi-square periodiogram. For this, the circadian period was calculated for the days before (τ_1) and after (τ_2) the light pulse respectively. The activity data of first two days after the light pulse was excluded to allow the transients to fade away from these analyses (circadian period and activity count). Light was provided by a compact cool fluorescent lamp (CFL; 14-watt; model 32B22 BC; Philips, Calcutta, India). The constant dim light (LL_{dim}) was provided at 0.3 lux (bulb 3.8V, 0.30A; Eveready Industries, Calcutta, India). Radiometer (model no. Q203; Macam Photometrics, Scotland) was used to measure the light intensity. Food and water were provided ad libitum.

The Student's *t*-test (unpaired *t*-test) was used for data analysis. The circadian period and day/night activity count were compared in both light intensities with the same or different time points. Significance was taken at $P < 0.05$. The statistical analysis was performed using Graph Pad Prism (v. 3) software program.

Results

Six representative actograms are shown in figures 1A, B and C, left panel: (i and ii). The weaver birds are diurnal and their activity was seen confined only to the daytime. The birds under 12L:12D synchronized their activity with the lights onset and offset. Except for individual variations, the general trend of activity was similar in all the birds under 1000 lux (groups 1, 2 and 3) and 10 lux (groups 4, 5 and 6) but intensity dependent effects were seen on the amplitude of activity; being higher in high intensity groups (1, 2 and 3) [Fig. 1A, B and C, left panel: (i and ii)]. The mean activity was significantly high ($P < 0.05$) in group 3 (1000 lux) under LD condition than in groups 6 (10 lux) [Fig. 1C, Middle panel: total activity during day and night activity (movement/12 h) (a)]. Under constant dim light (LL_{dim}), locomotor activity was diminished in all birds under both the light intensities (1000 and 10 lux) but the noticeable response occurred in groups 4 and 6 (10 lux) under LL_{dim} ; these groups showed greater activity as compared to groups 1 and 3 (1000 lux) [Fig. 1A and C, Middle panel: total activity during CT 0-12 and CT 12-24 activity (movement/12 h) (b and c)]. So, the activity under LL_{dim} indicate that the prior exposure to bright (1000 lux) and dim light (10 lux) intensities modulate the locomotor activity of weaver bird under constant illumination.

There was no significant effect of pretreatment with bright or dim light intensities on free running periods before (τ_1) and after (τ_2) the CT 12 light pulse in groups 1 and 4 [pre-pulse; group 1 (1000 lux), $\tau_1 = 24.10 \pm 0.22$ h, group 4 (10 lux), $\tau_1 = 24.32 \pm 0.29$ h and post-pulse; group 1 (1000 lux), $\tau_2 = 23.63 \pm 0.22$ h and group 4 (10 lux), $\tau_2 = 23.63 \pm 0.29$ h] [Fig. 1A, Right panel: circadian period (e and f)]. The free-running circadian period of group 2 (1000 lux) was increased [pre-pulse (τ_1) = 23.90 ± 0.19 h and post-pulse (τ_2) = 24.21 ± 0.21] after light pulse at CT 17, but statistically there was no significant change found in before and after the CT 17 light pulse in groups 2 (1000 lux) and 5 (10 lux) [pre-pulse (τ_1) = 24.18 ± 0.19 h and post-pulse (τ_2) = 23.35 ± 0.38] ($P = 0.5108$) [Fig. 1B, Right

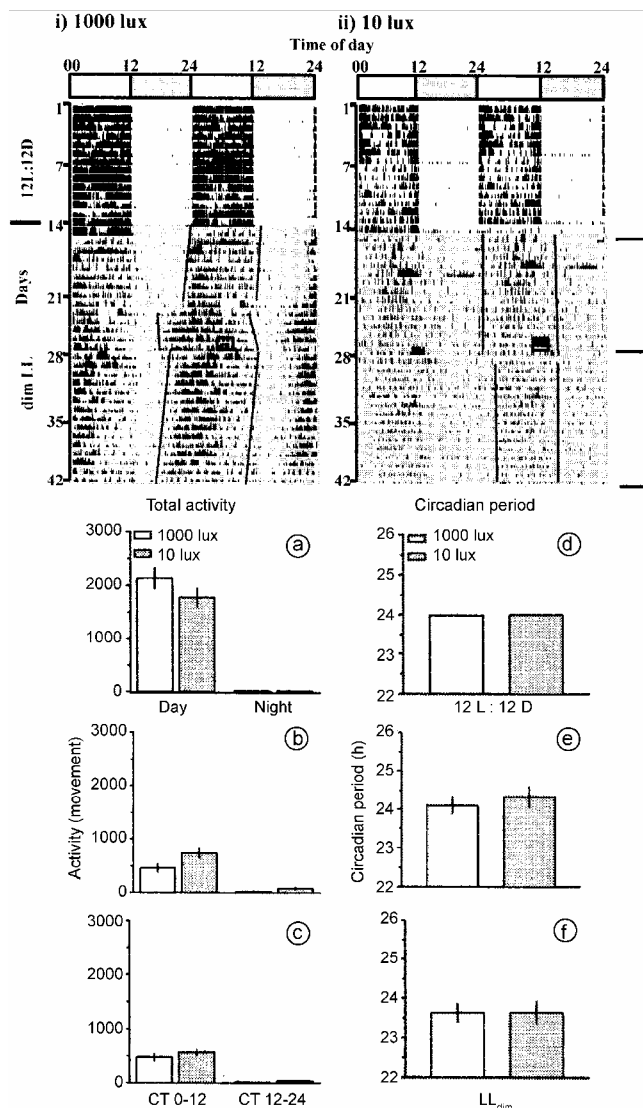


Fig. 1A—Left panel: Representative double plot of actograms of male Indian weaver bird exposed to 1000 (i) or 10 lux (ii) light intensity under 12L:12D and LL_{dim} with a 2 h light pulse at CT 12. Middle panel: total activity during day and night (a), circadian time 0-12 and 12-24 h (b) and (c) activity under LD and LL_{dim} for each 10 days (mean±SE activity counts/ 12 h) respectively. Right panel: Circadian period (τ) (mean ±SE) plotted over ten days under 12L:12D (d) and LL_{dim} (e) and (f).

panel: circadian period (e and f)]. There was no significant change observed in both groups [groups 3 (1000 lux) and 6 (10 lux)] of CT 20 light pulse during prior and after the light pulse [pre-pulse (τ_1); group 3 (1000 lux) = 24.25 ± 0.30 and group 6 (10 lux) = 24.04 ± 0.09] [post-pulse (τ_2); group 3 (1000 lux) = 24.22 ± 0.49 and group 6 (10 lux) = 24.16 ± 0.18] ($P = 0.3661$) [Fig. 1C, Right panel: circadian period (e and f)].

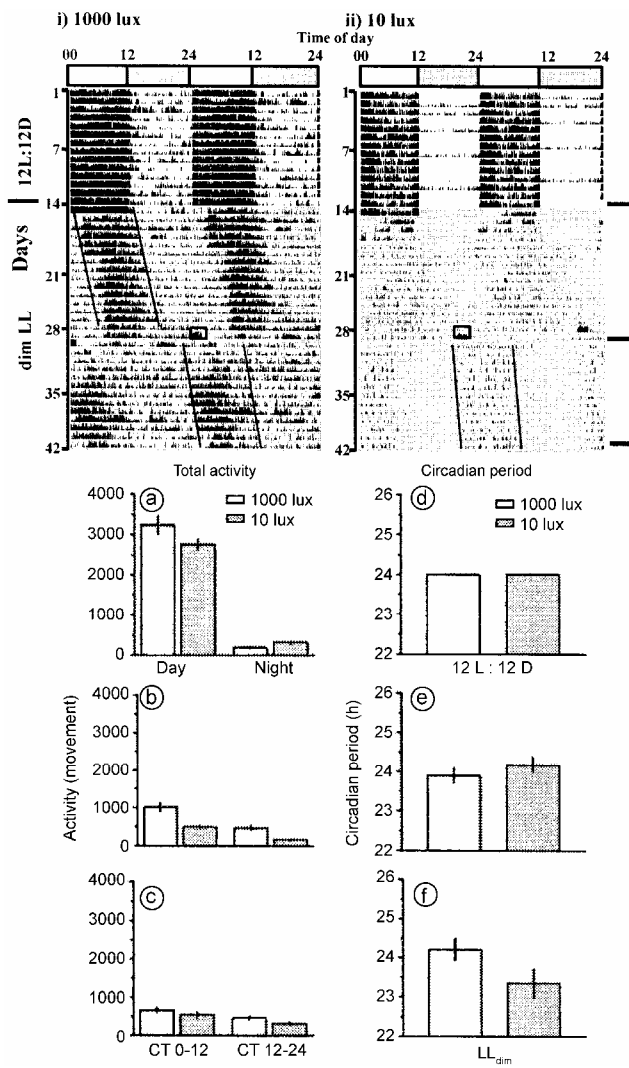


Fig. 1B—Left panel: Representative double plot of actograms of male Indian weaver bird exposed to 1000 (i) or 10 lux (ii) light intensity under 12L:12D and LL_{dim} with a 2 h light pulse at CT 17. Middle panel: total activity during day and night (a), circadian time 0-12 and 12-24 h (b) and (c) activity under LD and LL_{dim} for each 10 days (mean±SE activity counts/ 12 h) respectively. Right panel: Circadian period (τ) (mean ±SE) plotted over ten days under 12L:12D (d) and LL_{dim} (e) and (f).

Light pulses at different time points (CT 12, CT 17 and CT 20) under LL_{dim}, caused differential phase shifting responses in circadian behaviour of weaver birds. A single pulse of 2 h at CT 12 led to the delay in onset of activity in both the groups (1 and 4), however, there was no effect of pretreatment of different light intensities on phase shift [group 1 (1000 lux) -0.86 ± 0.39 h; and group 4 (10 lux) -1.40 ± 0.73 h]. After the light

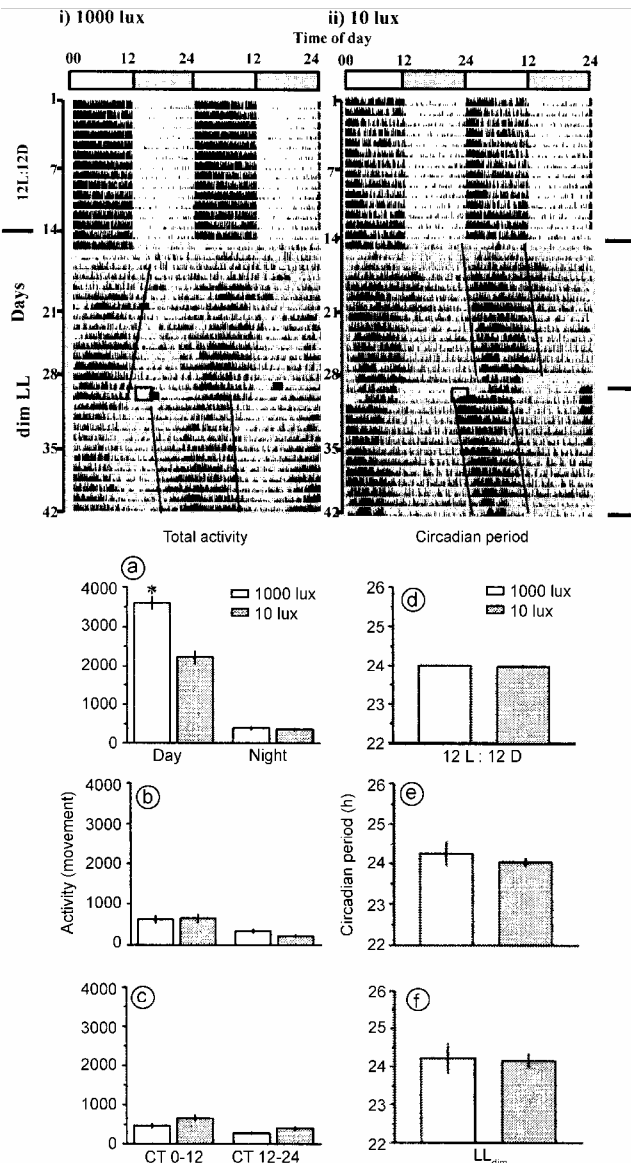


Fig. 1C—Left panel: Representative double plot of actograms of male Indian weaver bird exposed to 1000 (i) or 10 lux (ii) light intensity under 12L:12D and LL_{dim} with a 2 h light pulse at CT 20. Middle panel: total activity during day and night (a), circadian time 0-12 and 12-24 h (b) and (c) activity under LD and LL_{dim} for each 10 days (mean $\pm$ SE activity counts/ 12 h) respectively. Right panel: Circadian period (τ) (mean $\pm$ SE) plotted over ten days under 12L:12D (d) and LL_{dim} (e) and (f). Asterisks (*) indicates the significance on difference at $P < 0.05$.

pulse at CT 17, both groups (2 and 5) showed large delay in phase shift [(group 2 (1000 lux) - 3.61 ± 0.15 h and group 5 (10 lux) -3.86 ± 0.12 h)] and a single 2 h light pulse at CT 20 led the advance in onset of activity in both the groups [group 3 (1000 lux) 0.66 ± 0.12 h; group 6 (10 lux) 0.10 ± 0.01 h] (Fig. 2).

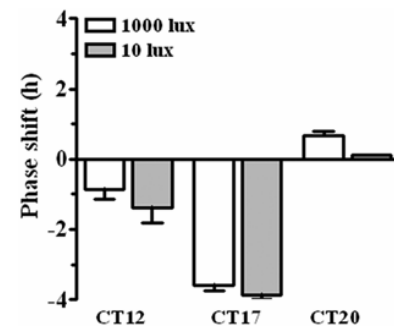


Fig. 2—Representative plot of phase shift (mean $\pm$ SE) after CT 12, CT 17 and CT 20 light pulses under LL_{dim}.

Discussion

Indian weaver bird is a diurnal species as its activity is confined to the light phase¹⁷. The birds were entrained to 12L:12D. Similar finding was reported by Hau and Gwinner on house sparrows under different light intensities (100 lux : 0.3 lux or 13 lux : 0.3 lux) under 12L:12D photoperiod^{18,19}. The activity was found to be light intensity dependent as lesser activity was noticed in birds exposed to 10 lux light intensity as compared to birds under 1000 lux. But under LL_{dim}, activity counts were more in groups 4 and 6 (10 lux) as compared to groups 1 and 3 (1000 lux). So, the endogenous circadian clock of weaver bird undoubtedly recognized the strength of light stimulus. Earlier study on Syrian hamsters exposed to LD cycles with gradual transitions in the light intensity demonstrated the entrainment, but when the hamsters were exposed to LD cycles with abrupt transitions in the light intensity (LD cycle of rectangular type), only 40% of the hamsters showed entrainment²⁰.

Study on wild rabbits suggested that the onset of locomotor activity depends on the intensity and colour of light in an artificial light-dark regimen²¹ and daily colour variation in the arctic sky in a polar summer season was able to entrain the activity of diurnal birds²². In contrast, weaverbirds exhibited circadian nature of their locomotor activity rhythm in dim light (LL_{dim}) and all birds free-ran with circadian period (tau) longer or shorter than 24 h.

Birds demonstrated difference in phase shifting response after the 2 h light pulses (1000 lux) at CT 12, CT 17 and CT 20 under LL_{dim}. The minimum phase delayed response was found in both groups which received pulse at CT 12 and the maximum phase delayed response was observed in both groups which received pulse at CT 17. But the advance phase shift was observed in both groups of CT 20 pulse. Thus, light pulses affect the onset of locomotor

activity depending upon their time (Fig. 2). The direction of the phase shift is a species-specific response. Eskin²³ have reported on sparrows, light pulses at beginning in the late subjective night (CT 16-CT 23) showed the large phase advances where as large phase delays observed when light pulse administered at late subjective day or early subjective night (CT 06-CT 14). In other finding suggested that the largest advance phase shift occurred during the late subjective night (CT 18-CT 00) and short delayed phase shift reported during the 4 h light pulse in early subjective night in sparrow²⁴.

Binkley and Mosher²⁵ determined the effect of two light pulses (doublets) in instantaneous resetting of the circadian clock. They maintained the birds under 12L:12D (800 lux) for 2 weeks and then transferred under constant darkness for exposure of either one 4 h light pulse or two 4 h light pulses (doublets). They suggested that the phase shift response to the second light pulse could be elucidated by assuming that the endogenous clock is reset by the initial (first) light pulse before the second pulse begins.

In cray fish the light alongwith low temperature produced opposite effects on the direction of the phase shift in the activity rhythm²⁶. The advance and delay phase shifts produced by low temperatures at dawn and dusk may protect the crayfish from unfavourable low temperatures depending on the season. A study on *Hipposideros speoris*, demonstrated that white light pulses of 15 min duration and 1000 lux intensity and of different spectral qualities evoked PRCs with deviant responses during the subjective night (CT 14 to CT 24) phases²⁷. Binkley and Mosher¹¹ reported the timing of the phase response curve and its amplitude was determined by the prior treatment of photoperiod in sparrow.

Pandey and Bhardwaj¹⁵ have shown that the circadian system of weaver bird is responsive to light illumination by synchronization and phase inversion of activity rhythm in response to changes in the light illumination under artificial (LD cycles) laboratory condition. The results of the present study showed that the light intensity affects circadian activity pattern of Indian weaver bird. However, light pulses at different circadian time (CT) point in LL_{dim} change phase and period length of circadian rhythm.

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