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The presence/absence of conspecifics modulates the circadian locomotor activity and body mass in spotted munia (*Lonchura punctulata*)

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ABSTRACT

Biological clocks regulate the behavior and physiology of animals by tracking the local time using diverse time cues. Social cues are relevant in studying the behavior of gregarious animals, but these cues have not been widely studied in birds. Temporal information for circadian timekeeping is socially communicated through visual, physical, olfactory, and auditory means. We examined the efficacy of pulsatile social interactions on locomotor activity and its associated characteristics such as distribution profile of rest and activity, total counts, activity duration, phase shift in activity onset, and circadian periodicity in spotted munia. Besides, we analyzed the effect of such social interactions on their body mass. Spotted munia exhibited phase shift in the onset of activity when subjected to social isolation, but these cues could not affect their circadian periodicity. In Pair as well as in Group, social isolation led to increased activity and activity duration, and decreased body mass in guests relative to the host bird. Our results suggest that the circadian rhythm of locomotor activity in spotted munia is quite sensitive to socialization and isolation, and isolation is detrimental for the birds. Consistent with these observations, the decline in body mass revealed the physiological consequences of social isolation on spotted munia.

Introduction

Living beings exhibit several daily rhythms such as locomotor activity rhythm, feeding rhythm, temperature cycle, melatonin profile, etc. under the control of circadian clocks, that in turn are synchronized by environmental factors; the light-dark cycle is the predominant one (Maury et al. 2010) in most species. Biological clocks regulate the behavior and physiology of animals by tracking the local time and using diverse time cues (Kumar 1997; Rani et al. 2009; Singh et al. 2010; Sur et al. 2020). This clock-centric regulation allows precise anticipation of the rhythmic changes in the environment, which is useful for the temporal distribution of locomotor activity, foraging, preening, vocalizations, and other behavioral practices in a day (Cassone 2014; Challet 2019; Kuhlman et al. 2018; Wang et al. 2012). These clocks per se are endogenous, self-sustained, and free-run in a constant environment (Aschoff 1981). The non-photic stimuli such as social cues, provide a social environment where the rhythmic signal is produced by the sender and received by the receiver. Social cues activate the mechanisms that are capable of sensing rhythmic sensory stimuli that primes the generation of

coordinated behavior leading to complex feedback loops between rhythmic information production and detection (Phillips-Silver et al. 2010). Among the higher vertebrates, several avian and mammalian species are social by nature.

Different animals respond differently to social cues, ranging from no effect on biological rhythm (Gattermann and Weinandy 1997; Kleinknecht 1985; Knadler and Page 2009; Refinetti et al. 1992) to significant effect. Recurrent social interactions have been found to function as *Zeitgeber* for the circadian clocks (Bessa et al. 2018) and for individuals confined to timeless environments, and these are among the crucial and probably the only source of temporal information (Crowley and Bovet 1980; Goel and Lee 1997; Levine et al. 2002; Marimuthu et al. 1981; Menaker and Eskin 1966; Viswanathan and Chandrashekaran 1985). Social cues are known to be effective in the house sparrow for entrainment by sound (Menaker and Eskin 1966) and among mammals, in beavers, for mutual synchrony of the circadian activity rhythm during winters when information about the light-dark cycle is lacking (Bovet and Oertli 1974), in

deer mice too for mutual synchronization of the activity rhythms of individuals by the presence or absence of conspecifics (Crowley and Bovet 1980; Marimuthu et al. 1981; Mrosovsky 1988; Viswanathan and Chandrashekaran 1985). Among invertebrates, a study on *Drosophila* suggests that they can adjust their circadian rhythm of locomotor activity upon receiving signals from conspecifics (Levine et al. 2002), while in honey bees, it is found that potential social synchronization can even dominate the entrainment by light (Fuchikawa et al. 2016). However, the role of social cues in synchronizing the circadian rhythms between or among individuals who are else isolated from other periodic environmental cues is relatively less explored (Knadler and Page 2009), and the mechanism by which social cues regulate activity behavior is also understated. To our surprise, the role of social cues in modulating the characteristics of the circadian rhythm of locomotor activity under constant conditions has hardly been studied in an avian model (but see; Menaker and Eskin 1966).

Relatively fewer studies have been conducted to assess the impact of social isolation on animals. A study on mice has revealed that social isolation increases metabolic demands (van Leeuwen et al. 1997), while Levine et al. (2002) have shown that *Drosophila* undergoes a phase shift in its locomotor activity under the influence of social cues. Besides, a study on migratory redheaded bunting (Singh et al. 2010) suggests that host and guest birds (isolates) had differences in activity upon isolation. This study examined the effect of the presence of a conspecific on survival benefits in a food-restricted environment created by limiting food availability. It was found that the presence of a conspecific reduced the mortality and enhanced the circadian performance of the individuals comprising the pair. Also, a study on human subjects suggests that total activity counts were lower in isolated individuals relative to non-isolated subjects (Schrempft et al. 2019). However, the consequences of social isolation on a resident non-photoperiodic bird are unknown. Communal regulation of the circadian rhythm by conspecifics can be an effective way of conveying time information (Bovet and Oertli 1974; Crowley and Bovet 1980; Gwinner 1966). Living in a group assures animals of many benefits (Krause and Ruxton 2002) such as efficient thermoregulation, food gathering, mate selection, parental care, escaping from predatory attack, and protection by the community members. But group living requires the synchronization of individuals' rest-activity behavior which has hardly been studied; particularly in birds; hence,

Paul et al. (2015) have suggested studying the social regulation of circadian clocks of cohabiting animals.

Birds offer admirable opportunities to study grouping, since closely related species, that are almost alike in most features of behavior and ecology, still exhibit severe alterations in the range of sociality i.e., from pairs to large flocks. The finch family Estrildidae is exceptional in this regard. The majority of estrildid species form small groups of about 6–12 birds when they are not breeding, and then loosely distribute themselves for nesting (Goodson and Kingsbury 2011). Spotted munia is particularly a good experimental species for addressing our question because, firstly, circadian rhythms of locomotor activity are well-characterized in this species (Agarwal et al. 2017), secondly, it is a non-photoperiodic bird (Budki et al. 2014) so it responds well under constant light conditions and thirdly, these birds live in flocks and typically form small groups (Ali and Ripley 1999; Goodson and Kingsbury 2011; Pradhan et al. 2016) hence, studying their daily behavior in a social context holds rationale and can contribute significantly to the field.

Therefore, the present study aims to examine the effect of socialization and isolation on locomotor activity and body mass of spotted munia, housed initially under LD cycle and then under constant dim light conditions. Under these light regimens, the birds were housed in an individualistic or clubbed manner, and they were subjected to socialization and isolation protocol. In this context, we have tried to answer certain questions pivoted around the circadian characteristics; (i) if the social cues maintain circadian periodicity of Pair and Group housed birds similar to that of Single birds, (ii) whether social interactions affect the phase shift in the activity onset, (iii) how the social isolation affects characteristics of locomotor activity among host and guest(s) birds, (iv) if the activity duration gets affected by pulsatile social interactions, and (v) how the social isolation, post socialization affects the body mass of spotted munia. We consider that our study would unravel the underpinnings related to the efficacy of social cues under constant light conditions and expect that social isolation would be detrimental for the birds whereas socialization would be rewarding for them.

Materials and methods

Animal and maintenance

The experiment was done in the non-breeding season on spotted munia (*Lonchura punctulata*), all procured from nature in the last week of December from a flock of birds (*n* = 50) using a mist net. At that time, the average

temperature was 16°C, and the daylength was ~10.5 L:13.5D in nature. All birds were naive to the experimental conditions since they were captured from the wild. As per Chandola and Thapliyal (1978), spotted munia begins gonadal recrudescence in nature soon after the summer solstice, and remains fully active up to October/November. Gonads regress before the winter solstice. The birds were in adult condition as confirmed by the lack of any juvenile plumage (Moynihan and Hall 1955). Spotted munia is a ~10 cm long subtropical passerine finch belonging to the family Estrildidae, order Passeriformes (Ali and Ripley 1999). They respond well under laboratory conditions in captivity (Ruchi and Malik 2015; Srivastava et al. 2015). Being social, they are strongly inclined to stay together and tend to intimidate the behavior of their companions (Moynihan and Hall 1955).

After procurement, the birds were brought to the laboratory and initially kept in an outdoor aviary (size = 3.0 × 2.5 × 2.5 m) for a week under natural daylength (NDL) and temperature conditions as stated above (Agarwal et al. 2017; Ruchi and Malik 2015). Thereafter, they were moved to an indoor aviary (size = 2.5 × 2.0 × 1.6 m) under controlled laboratory conditions for ~2 weeks. After acclimation, they were selected randomly (Bhatt and Sengupta 1991) to be housed in activity cages (size = 60 × 45 × 35 cm) placed inside the photoperiodic chambers (size = 71 × 67 × 48 cm) provided with compact fluorescent lamps (14 watts; Bajaj Electricals Limited, India) under controlled ambient conditions of light (12 L:12D photoperiod; controlled by electronic timer) and temperature (23 ± 2°C). Consistent with previous studies (Agarwal et al. 2017; Singh et al. 2010), these birds did not get stress as observed by their behavior (for example, daily activity, lack of a distress call, or posture) when moved indoors or when housed in activity cages; birds make a distress call under stressful conditions such as predator attacks or removal from mist nets (Greig-Smith 1982; Rohwer et al. 1976). Each activity cage was equipped with two perches, a food cup, a water cup, and a Passive Infrared Motion Sensor (Conrad Electronic, Germany, Haustier PIR- Melder) mounted from outside through which data was obtained via computerized recording of locomotor activity. Briefly, each IR sensor was connected to a separate channel of the computerized data recording system that collected locomotor activity of the bird detected as hopping from one perch to the other or actively moving from one side of the perch to the other, inside its cage in 5 min bins by the KitAnalyze Software Version 1.06 Stanford Software Systems, USA (Malik et al. 2004). Each activity cage was acoustically isolated from the other and the photoperiodic chamber

used to remain closed unless otherwise opened for replenishing food and water and for cleaning. Food (a mixture of seeds of *Setaria italica* and *Oryza sativa*) and water were provided *ad libitum*. Twice a week, a supplementary feed was also provided (Singh et al. 2010).

Experimental protocol

The study was carried out at the Department of Zoology, University of Lucknow, Lucknow (26°55' N, 80°59' E), India, under the research project approved by University Grants Commission, New Delhi, India [36–267/2008 (SR)], and following the ethical guidelines for animal experimentation. For the experiment, birds (n = 36) were randomly subjected to either of the three kinds of housing conditions namely; Single (n = 1/cage), Pair (n = 2/cage), and Group (n = 3/cage). It is to be noted here that we have not found any sex-dependent bias in the locomotor activity pattern of these birds (our unpublished observation; Rani et al. is shown in Supplementary Figure S1. The data has been derived from five birds of either sex, for 7 days. The representative actogram of male and female spotted munia has also been shown in the supplementary figure .), so we selected the birds randomly; as also done for spotted munia by Bhatt and Sengupta (1991). Single birds were housed individually and did not undergo any social interaction protocol, and therefore served as control. However, the other two cohousing assemblies underwent social interaction protocol. Paired birds could have been male-male/female-female or male-female. The same holds true for Group housing condition. Since a Pair had two birds, the number of cohoused individuals was increased to three to form a Group that could be housed in the IR motion sensor equipped activity cages. We housed the birds as Single, Pair, and Group as we expected different effects of social interaction on the circadian activity output among a Pair of birds and a Group of birds when compared with the Single housed bird.

All the birds were initially kept in their respective socialized conditions (i.e., Single, Pair, and Group) in Social 1 (S1) episode under 12 L:12D photoperiod (L:D = 85 lux:0.04 lux) for 21 days, and then the LD cycle was replaced with constant light conditions LL_{dim} (0.04 lux) for the rest of the experiment (i.e., 54 days). The first 23 days under this constant light condition were considered as Social 2 (S2) episode, which was identical to S1 except for the lighting condition. Thereafter, these birds were separated and housed in discrete cages for 14 days; Isolation 1 (I1) episode. The bird which was transferred to another cage was called the guest and the remaining one, the host. At the time of transferring the guest bird,

the host bird was also handled similarly, except for the fact that it was kept back in the same cage while guest was moved to a dedicated new cage. For transferring purposes and refraining from handling stress, the birds were handled just at the beginning and at the end of isolation episodes that lasted for a couple of days (I1 for 14 days and I2 for 9 days), and not several times a day. Since all the birds were handled at the time of isolation (including the Single housed birds), handling at the time of isolation could not be considered a confounding factor for activity differences. Thus, the Pair had one guest and one host while Group had two guests and a host.

Subsequently, the birds were again socialized for 8 days in the host cages; Social 3 episode, followed by

separation for 9 days; Isolation 2 (I2) episode, similar to that in I1. So, there were three housing conditions (Single, Pair, and Group) and 5 episodes of social interaction (S1, S2, I1, S3, and I2) as shown in Figure 1. To summarize, S1 episode lasted 24/7 for 21 days, S2 for 23 days, I1 for 14 days, S3 for 8 days, and I2 for 9 days. Each bird passed through every episode for the specified number of days. During socialization, the same birds were clubbed together and during Isolations every time one bird served as the host and the other as guest(s) based upon their tag number, and hence, the pre-decided guest bird was moved to a new cage (but that remained same during both the isolations). It is to be noted here that each episode lasted for several days as stated above; it was not momentary for a few minutes or
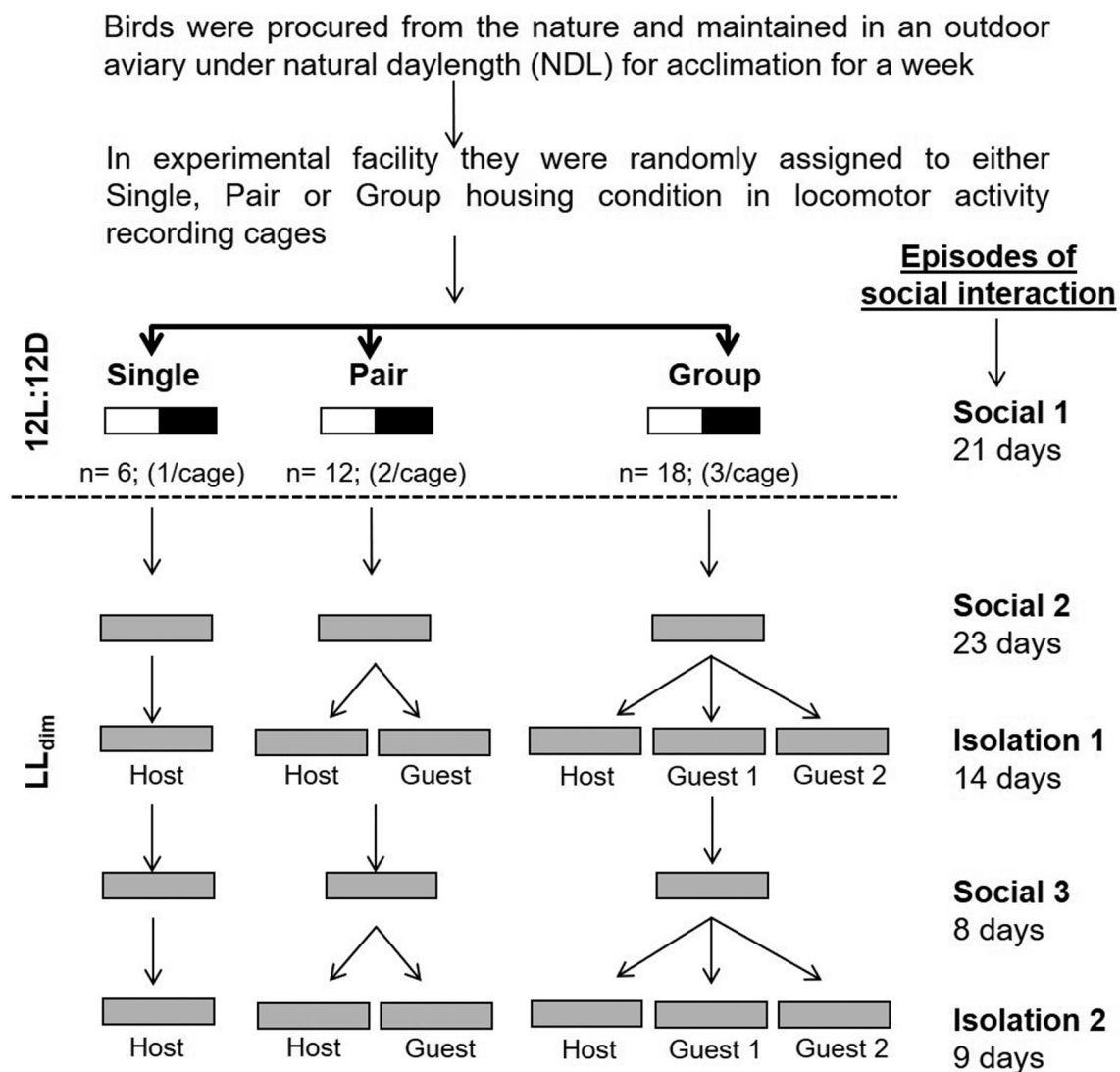

Figure 1. Spotted munia (*Lonchura punctulata*) after acclimation, were distributed into three housing conditions; Single, Pair and Group under 12 L:12D photoperiod (Social 1). After 21 days, LD cycle was replaced with constant light condition (LL$_{dim}$) for the rest of the experiment. Under LL$_{dim}$, for the first 23 days, birds remained cohoused like earlier (Social 2), followed by the separation of guest bird(s) from the host (Isolation 1) for 14 days. Thereafter, the birds were cohoused again with their respective partners (Social 3). After 8 days of cohousing, the guest bird(s) were finally isolated (Isolation 2) from their host and remained as such for 9 days.

few hours and constant exposure to fellow bird(s) was provided rather than momentary exposure except for the span of isolations. Also, on the day of the transition from one episode to another, a random time was chosen; e.g., separation at midday or socialization later in the day and vice-versa the next time.

To test the effect of pre-treatment, we decided to go for S3 and I2 also under constant dim light conditions. This was done: (i) to test if the results of S2 and I1 get replicated during S3 and I2 respectively (ii) to keep the number of days/conditions, and the sequence of episodes ambiguous to the birds and (iii) to observe if the effect of an episode changes with time. For birds to show their overt response, the five episodes of social interaction were assigned different time windows; in terms of number of days (S1–21 days, S2–23 days, I1–14 days, S3–8 days, and I2–9 days), so that firstly, the birds do not anticipate any upcoming regimen and the bias (if any) based on exposure for the equal number of days could be neglected and secondly, a quicker effect of isolation was expected based on the available literature. This experimental paradigm was preferred to get an explicit response from the birds upon unfixed socialization and isolation conditions. We recorded the body mass of all birds individually, at three time-points; initially while housing them in activity cages as Single, Pair, and Group in the LD cycle (LD_1 time-point), then at the end of the LD cycle (LD_2 time-point) and finally at the end of Isolation 2 episode under LL_{dim} (LL_{dim} time-point). To keep the host-guest isolation process quick and avoid unnecessary handling of the birds during the experiment, body mass was not recorded at every episode of the experiment; especially during isolation. However, at the end of Isolation 2, since the experimental schedule was being terminated, we recorded it. Body mass was recorded using a top pan balance to an accuracy of 0.1 g (Malik et al. 2004). For this, a rectangular cotton cloth bag was tare to zero, followed by gently putting the bird in it and placing it back upon the balance to record the body mass.

Other important facets of the study are: first, since we could not record the individual activity when the birds were together in a cage, as our activity cages were not equipped with transmitters, we decided to record their socialized activity to compare S1, S2, and S3 among themselves in a particular housing condition. For this, it was presumed that, if one episode of socialization would not be different from the other, i.e. if S1, S2, and S3 are indifferent to each other, that would suggest that birds being either highly synchronized or there was no synchronization at all between these episodes; whereas, if any difference is obtained among the episodes of socialization, that would be attributed to the events before or in between the socializations, i.e., LD cycle or episode of isolation. Second, our hypothesis and study centered around analyzing the effect of conspecific cues on daily activity and body mass of a social bird; therefore, our objective was to study the effect of socialization and isolation on a random population of spotted munia in the non-breeding season. We selected the non-breeding season for the experiment so that sex-influenced outcomes could be subsided. Taking this into account, the gonadal status and sex of the birds were not examined as gonads remained regressed during that time of the year (Chandola and Thapliyal 1978). Chandola et al. (1982) have reported that the reproductive cycle of spotted munia has a period length of 10 months under constant darkness or light. Budki et al. (2012) housed spotted munia under captivity for 28 months and found that the male reproductive cycle lasted for ~11 months, but the female reproductive cycle length was relatively larger ranging from ~ 10–13 months under artificial light conditions in the laboratory set up. The current study protocol involved 75 days in total, and since the birds were procured in the last week of December; so, as per the available literature, gonads remained probably small despite the sex.

Locomotor activity behavior assay

Daily activity records (actogram) for socializations and isolations were double plotted to illustrate a better graphical representation of activity behavior with each succeeding day recurring sideways and underneath. For all the housing conditions, locomotor activity behavior data was analyzed for five consecutive days; lying in the middle of the LD cycle for S1 and from the beginning of each transition under LL_{dim} for the rest of the episodes. Data from the five days obtained was used to plot time series of locomotor activity, rest and activity distribution profile, total activity/day, activity duration/day, and phase of onset of activity. The data was exported in an hourly bin to plot the time series of locomotor activity (which is activity-rest cycles of consecutive days). Next, we exported the data in 10 min bin size for better resolution to study the hourly distribution of rest and activity profile of birds for five days; where the period of inactivity was considered as rest (Buehler et al. 2009). To calculate this, the number of null values per bin in an hour was counted for the same five consecutive days. To obtain the value of rest in an hour, the number of null values/h was multiplied by 10 and then divided by 60. This analysis provided us with the rest profile, which when subtracted from 1 provided the values for the activity profile in a 24 h cycle for five days.

For average total counts, we summed up the total activity counts in a day and then calculated its mean for five days (Malik et al. 2004; Singh et al. 2012). To calculate the activity duration/day, we summed up the hourly activity duration for the day and then the average was taken for five days for graphical representation. To calculate the phase difference in the onset of activity at S1, the time of lights on was taken as the reference point whereas, for other episodes, the difference between consecutive days provided the value of phase shift in the onset of activity following which its average was taken to nullify the possible prominent effect on day 1. The endogenous period of the locomotor activity was obtained by Chi-square periodogram analysis (Agarwal et al. 2017; Budki et al. 2014). This program automatically normalizes the plots to the maximum point, which shows the corresponding period mentioned above it. If the value crosses the horizontal yellow line on the graph (confidence limit) then it represents rhythmicity whereas if the value remains below the line, then that indicates arrhythmicity. During the data analysis, locomotor activity data had not been normalized because the study was done considering a random population of social birds, and had the data been normalized to a set scale, it would have diluted the effect of overt social interactions with respect to the housing condition. Although, it seems obvious for the activity counts for Group to be higher than Pair and Single, but, other than that we are showing other aspects of activity behavior such as phase shift and activity duration that reveal how the housing condition and episode of social interaction affected the expression of behavior.

Data analysis

Acquisition of actograms, determination of the phase of activity onset, and Chi-square periodogram analysis was conducted using The Chronobiology KitAnalyze software Version 1.06 Stanford Software Systems, USA. Two-way analysis of variance (repeated measures by column) (2-way RM-ANOVA) followed by the Bonferroni posttests if ANOVA indicated a significant difference, tested the effect of housing condition (factor 1), the episode of social interaction or time of the day; as appropriate (factor 2), and their interaction (factor 1 × factor 2) on different circadian variables. To study how different episodes of socialization are diverse from each other in a particular housing condition, we compared their mean by 1-way RM-ANOVA followed by the Newman-Keuls Multiple Comparison test. Whereas to study the effect of isolation, the mean values of host and guest were compared by Student's unpaired t-test in Pair, while in Group, the mean values of the host,

guest 1, and guest 2 were compared by 1-way ANOVA without repeated measures followed by Newman-Keuls Multiple Comparison test. 1-way ANOVA without repeated measures followed by Newman-Keuls Multiple Comparison test was also used to compare Single, Pair, and Group housed birds at S1, S2, and S3 while Single, Pair host, and Group host at I1 and I2. Significance was taken at $p < 0.05$. Since Single birds served as control and were housed individually throughout the experiment, their episodic data corresponds to the same day and time as Pair and Group birds for comparison.

Effect sizes were estimated to assess the magnitude of the responses (Mishra et al. 2020); Eta squared (η^2) for one-way ANOVA [SS_{Effect}/SS_{Total}; where SS_{Effect} is the sum of squares of effect and SS_{Total} is the sum of squares of total], partial Eta squared (ηp^2) for two-way ANOVA [$SS_{Effect}/(SS_{Effect} + SS_{Error})$; where SS_{Error} is sum of squares of error] and Glass's delta [$(M1-M2)/S_{control}$, where M1 and M2 are the means of the sample for host and Guest, respectively, and $S_{control}$ is the standard deviation of the host; https://www.socscistatistics.com/effectsize/default3.aspx (George et al. 2021)] for Student's t-test. The effect sizes were calculated using Glass's delta instead of Cohen's d, since each group had different standard deviations. For ANOVA, a commonly used interpretation is to refer to effect sizes as small (0.01), medium (0.06), and large (0.14) (Lakens 2013), while for Student's t-test, the interpretation of effect size is small (0.2), medium (0.5), and large (0.8) (Cohen 1988). It is to be noted here that the effect size holds for consideration once the null hypothesis gets rejected. The data are presented in Figures 1–6 as mean ± SEM while statistics are shown in Tables 1–4. Supplementary Figures S2–6 shows the Chi-square periodogram obtained during the five episodes of social interaction for the three housing conditions. Supplementary Table S1 and 2 summarizes the frequency distribution of birds based on their period and phase of the activity under constant light conditions. Graphical designing and the statistical analyses of the data were conducted using GraphPad Prism software Version 5.01, California USA.

Results

The representative actograms of the Single, Pair, and Group housed birds (Figure 2) demonstrate that under S1, birds were active during the day and took rest during the night; however, on transfer to LL_{dim} (constant light condition), their locomotor activity got altered, and they started "free-running." During isolations, it is more evident that guests were more active than the host bird
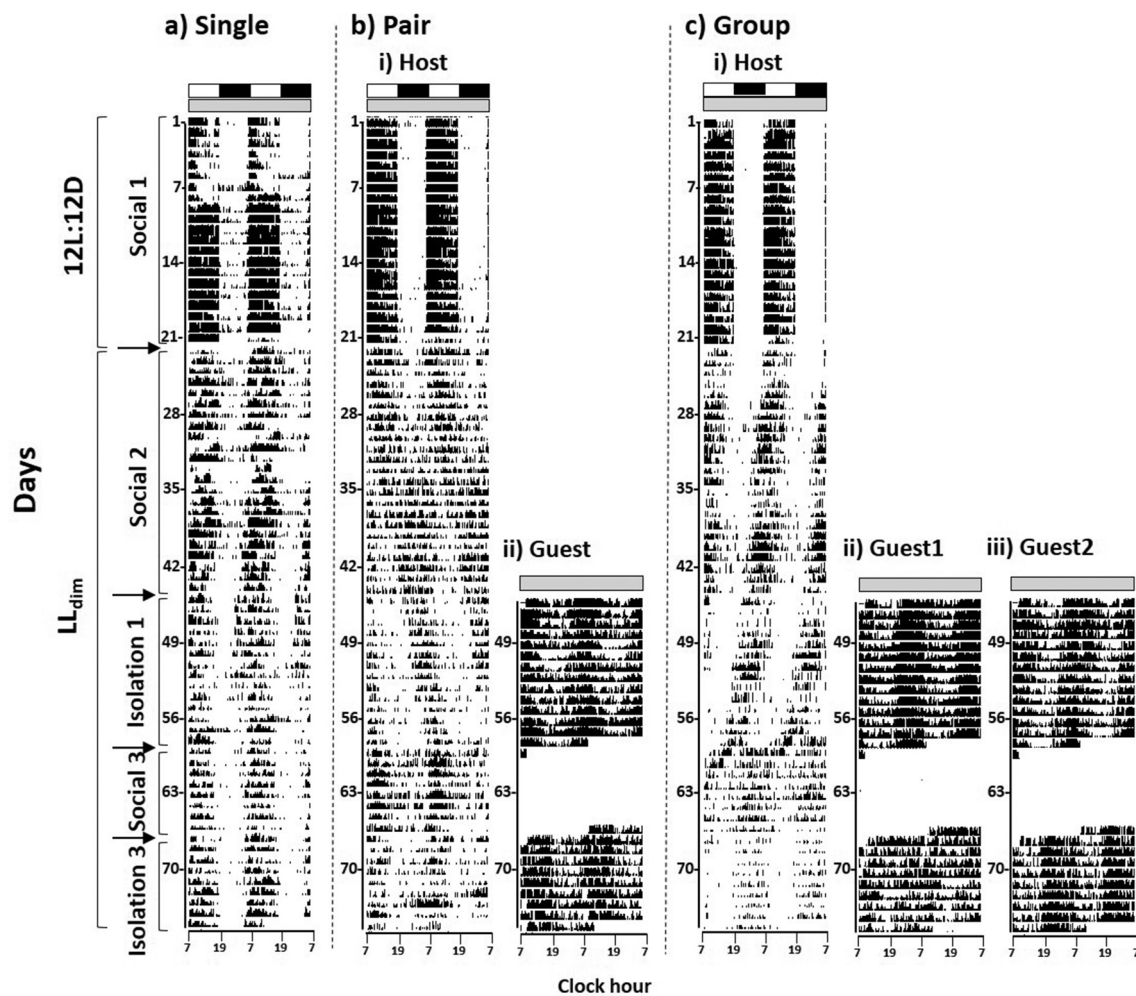
Figure 2. Representative double plotted actograms of spotted munia housed as Single (a), Pair (b), and Group (c) under 12 L:12D and LL$_{dim}$ photoperiodic conditions. Each episode of social interaction is marked along the actogram on the left side as Social 1, Social 2, Isolation 1, Social 3, and Isolation 2. Host (i), and guest(s) (ii) and (iii) are present along each other in both Pair (b) and Group (c). Horizontal bars above actograms represent light and dark condition.

in Pair as well as in Group, but the pattern of activity in Single housed birds seems to remain unchanged. In I2 also, Pair guest conceivably intensified its activity. Phase shift in activity onset could be observed in the pattern of locomotor activity with each passing day and at transitions from one episode to the next.

The time series of locomotor activity (Figure 3) shows the alteration in activity pattern over five days. Activity counts/h and the amplitude of activity decreased when birds were transferred from LD cycle to LL$_{dim}$ (see the scale on the Y-axis of graphs). Rest and activity profiles (Figure 4) show the hourly distribution of rest and activity in a 24-h cycle for five days. At every hour, the distribution pattern of rest and activity were mirror images of each other. Total activity counts (Figure 5) were calculated to quantitatively evaluate the total activity counts across a day, among the three housing conditions. Although it seems obvious for the

activity counts of Group to be higher than Pair and Single, mean ± SEM allocation of total activity per bird during S1 in Group (1211.42 ± 301.18) was comparable to both Single (2086.00 ± 385.09) and Pair (1125.00 ± 229.96), and surprisingly it was significantly low in Pair as compared to Single housed birds. Statistically, we found an overall significant effect of episodes of social interaction on total counts ($p < 0.05$; Table 3a). Activity duration/day (Figure 5) was calculated to quantitatively analyze the duration for which birds remained active in a daily cycle among the three housing conditions. We found an overall significant effect of episodes of social interaction and its interaction with the housing condition on activity duration/day ($p < 0.05$; Table 3a). Phase difference (Figure 5) was calculated to assess the duration of phase advance (+ve shift) or phase delay (−ve shift) in the onset of locomotor activity under the experimental condition. Statistically, we found a
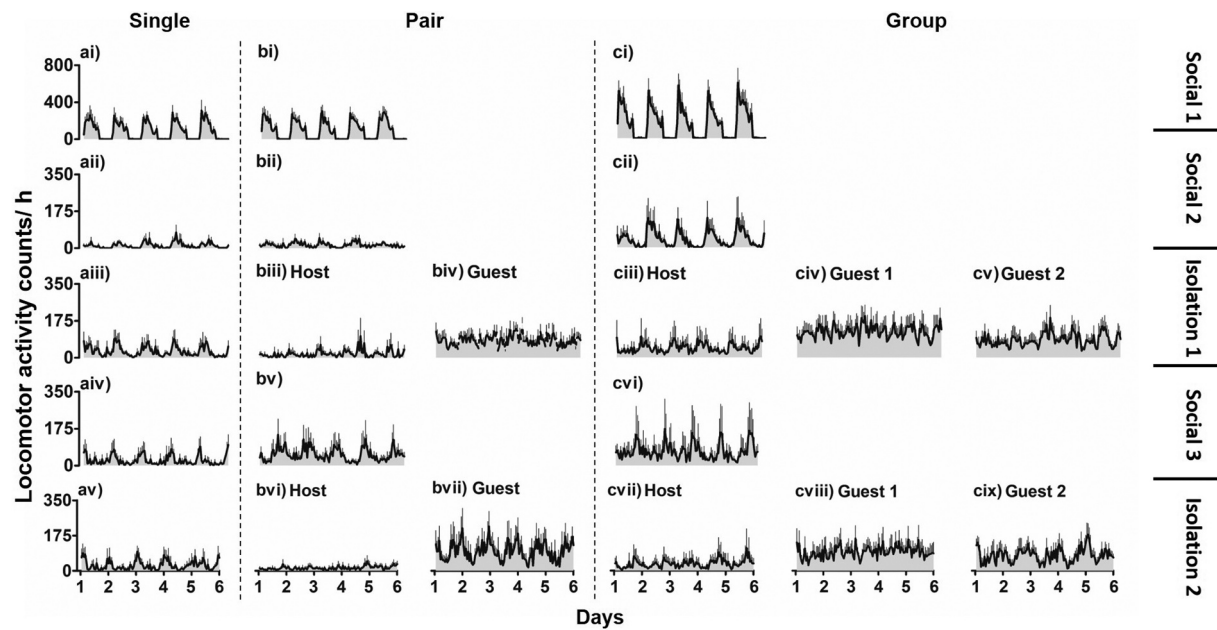
Figure 3. The time series of locomotor activity of Single (a), Pair (b), and Group (c) housed spotted munia under 12 L:12D and LL$_{dim}$ regime plotted over five consecutive days as mean (± SEM). Each episode of social interaction is demarcated along the graphs on the right side as Social 1, Social 2, Isolation 1, Social 3, and Isolation 2. Activity time series of host and guest(s) are present along each other in both Pair (b) and Group (c).
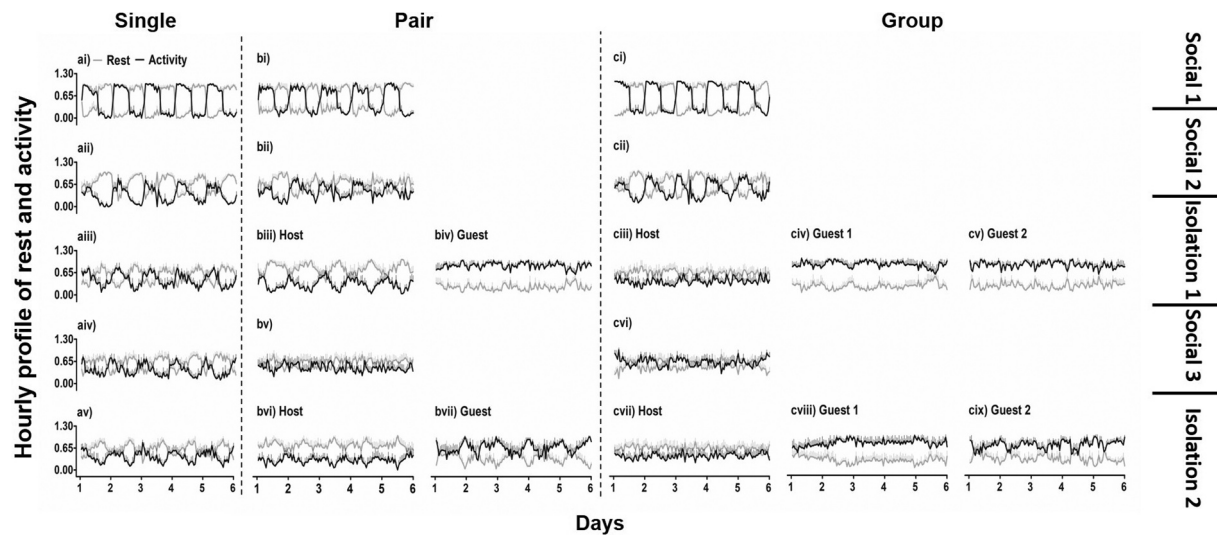

Figure 4. Hourly distribution profile of rest and activity of Single (a), Pair (b), and Group (c) housed spotted munia under 12 L:12D and LL$_{dim}$ regime plotted over five consecutive days as mean (± SEM). Each episode of social interaction is demarcated along the graphs on the right side as Social 1, Social 2, Isolation 1, Social 3, and Isolation 2. Rest and activity profile of host and guest(s) are present along each other in both Pair (b) and Group (c).

significant effect of episodes of social interaction and its interaction with the housing condition on the phase of activity onset ($p < 0.05$; Table 3a). Circadian periodicity (Tau τ) or the endogenous period of rhythm was calculated to assess the variation in the period length of the daily cycle in Single, Pair, and Group housed birds. Tau = T when the animal is under LD cycle, and entrained. We did not find any overall significant effect of any of

the factors and their interaction on circadian periodicity ($p > 0.05$; Table 3a).

Now, for ease of understanding the insights, the results have been divided into three sections; Section I: Episode-wise effect on activity behavior among Single, Pair, and Group housed birds; Section II answers if social housing affects the response to LL$_{dim}$ by showing comparisons among S1, S2, and S3 in a particular
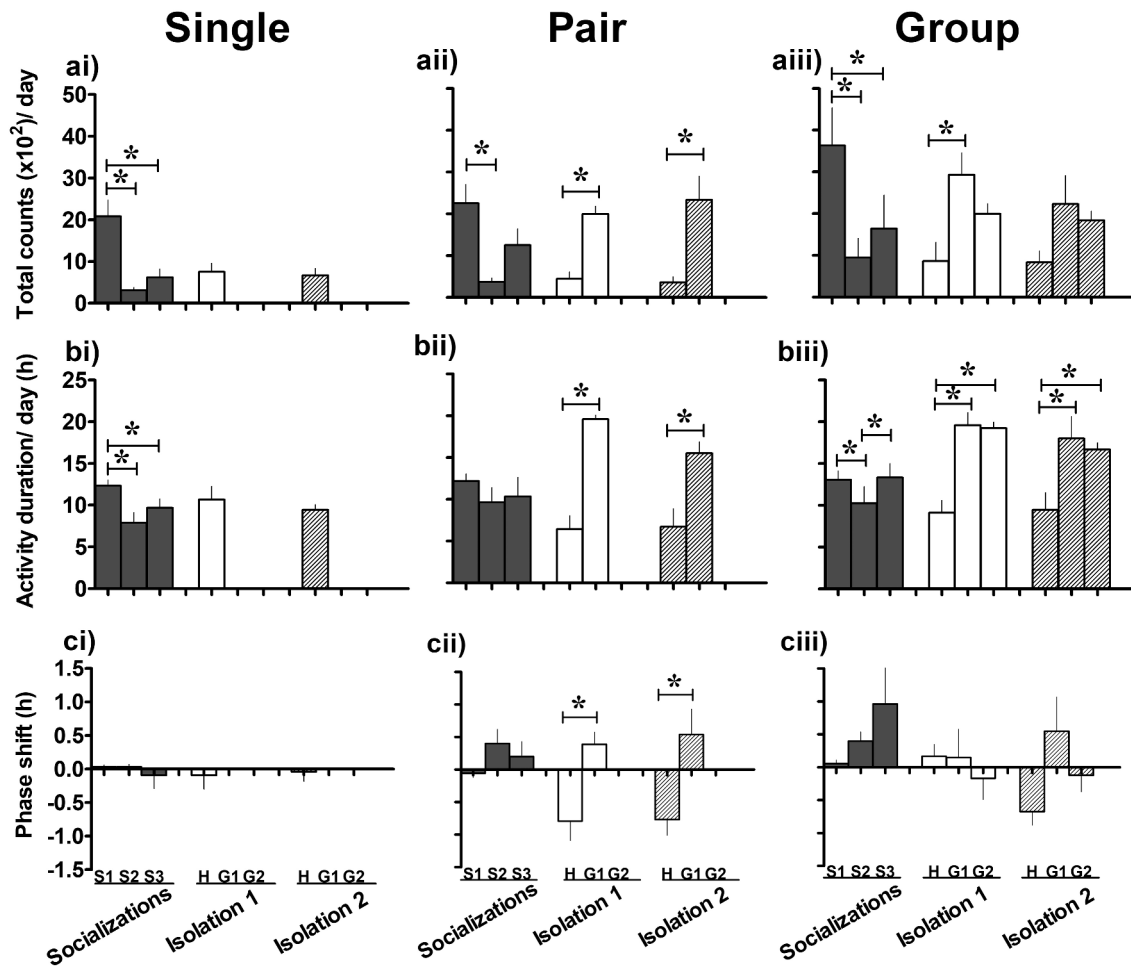
Figure 5. Total counts (ai-aiii), activity duration (bi-biii), and phase shift in onset of activity (ci-ciii) of Single, Pair, and Group housed spotted munia under social (S1, S2, and S3; grey bars), Isolation 1 (white bars) and Isolation 2 (hashed bars) conditions are shown as mean (± SEM). Host, guest 1 and guest 2 are represented as H, G1 and G2 respectively. *above bars indicate the point of significant difference.

housing condition and Section III: addresses if isolations affected the locomotor activity of Single/host birds and the guest birds post-socialization.

Section I: episode-wise effect on activity behavior among Single, Pair, and Group housed birds

Effect of Social 1 (S1) episode on locomotor activity characteristics

Time series of locomotor activity. Time series varied significantly when compared among the three housing conditions. A significant effect of the time of day and its interaction with housing conditions was found on the time series of locomotor activity ($p < 0.05$; S1 in Table 1a). This pattern of activity varied significantly between Single and Group (Figure 3ai and ci) and also between Pair and Group (Figure 3bi and ci).

Hourly distribution of rest-activity. We found a significant effect of the time of day and its interaction with the housing condition on the hourly distribution profile of rest and activity ($p < 0.05$; S1 in Table 2a). During S1, the distribution pattern of rest and activity varied significantly between Single and Group (Figure 4ai and ci) and also between Pair and Group (Figure 4bi and ci).

Total activity counts. Total activity counts were not affected by housing conditions at S1 ($p > 0.05$; Total activity counts; Social 1; in Table 3). It was indifferent among Single, Pair, and Group housed birds.

Activity duration. Activity duration remained unaffected by the housing conditions at S1 ($p > 0.05$; Activity duration; Social 1; in Table 3). Like total activity, it was also indifferent among Single, Pair, and Group housed birds.
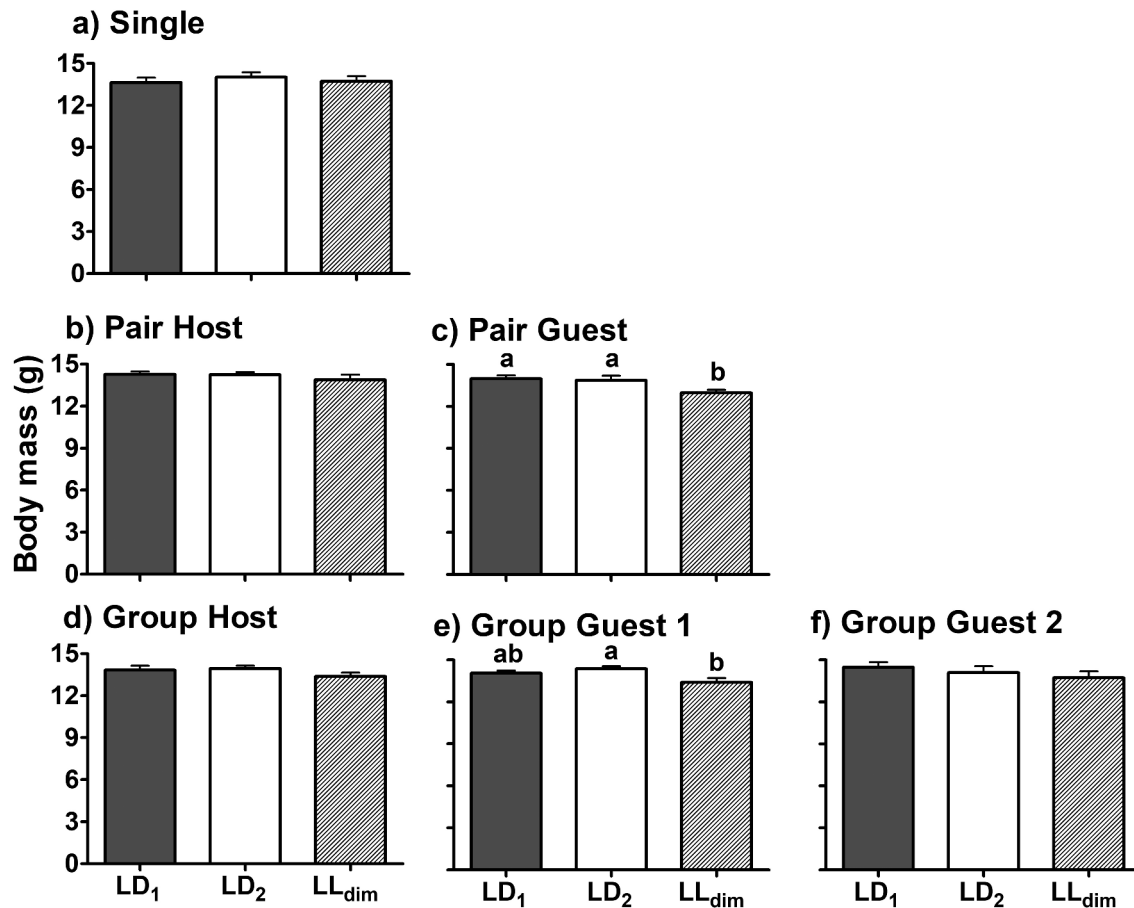
Figure 6. Body mass (g) of spotted munia housed as Single (a), Pair host (b), Pair guest (c), Group host (d), Group guest 1 (e), and Group guest 2 (f) at three time-points (LD_1, LD_2, and LL_{dim}) are shown as mean (± SEM). Dissimilar and similar alphabets above the bar indicate point of significant and insignificant difference respectively.

Phase shift in activity onset. Like total activity and activity duration, phase shift in the onset of activity also remained unaffected by the housing conditions at S1 ($p > 0.05$; Phase shift; Social 1; in Table 3). It was indifferent among Single, Pair, and Group housed birds.

Circadian period. Circadian period also remained unaffected by the housing conditions at S1 ($p > 0.05$; Circadian period; Social 1; in Table 3). It was indifferent among Single, Pair, and Group housed birds.

Effect of Social 2 (S2) episode on locomotor activity characteristics
Time series of locomotor activity. Like S1, at S2 also, the locomotor activity pattern was significantly affected by the time of day and its interaction with housing condition ($p < 0.05$; S2 in Table 1a). The pattern of activity varied significantly between Single and Group

(Figure 3aii and cii) and also between Pair and Group (Figure 3bii and cii).

Hourly distribution of rest-activity. Like S1, at S2 also there was a significant effect of the time of the day and its interaction with housing condition on the distribution profile of rest and activity ($p < 0.05$; S2 in Table 2a). During S2, the pattern of distribution varied between Single and Group (Figure 4aii and cii).

Total activity counts. Total activity counts were not affected by housing conditions at S2 ($p > 0.05$; Total activity counts; Social 2; in Table 3). It was indifferent among Single, Pair, and Group housed birds.

Activity duration. Activity duration remain unaffected by the housing conditions at S2 ($p > 0.05$; Activity duration; Social 2; in Table 3). Like total activity, it was also indifferent among Single, Pair, and Group housed birds.

Table 1a. Results of two-way RM ANOVA analyzing the effects of housing condition, the time of day, and their interaction on locomotor activity time series of spotted munia under different episodes of social interaction; Social 1, Social 2, Isolation 1, Social 3, and Isolation 2. Effect size estimates showing the magnitude of response are presented as partial Eta squared (η_p^2). F and p values in bold indicate points of significant difference. For statistical significance, the alpha was set at 0.05.

	Two-way ANOVA statistics								
The episode of social interaction	Housing condition			The time of day			Interaction (Housing condition x The time of day)		
	F value	p value	η_p^2	F value	p value	η_p^2	F value	p value	η_p^2
Social 1	$F_{(2, 1785)} = 1.844$	0.1922	0.1002	$F_{(119, 1785)} = 27.26$	<0.0001	0.6450	$F_{(238, 1785)} = 1.332$	0.0011	0.1508
Social 2	$F_{(2, 1785)} = 1.638$	0.2272	0.0978	$F_{(119, 1785)} = 3.755$	<0.0001	0.2002	$F_{(238, 1785)} = 1.263$	0.0064	0.1441
Isolation 1	$F_{(5, 3570)} = 8.530$	0.0001	0.2586	$F_{(119, 3570)} = 1.355$	0.0070	0.0432	$F_{(595, 3570)} = 0.6850$	1.000	0.1025
Social 3	$F_{(2, 1785)} = 0.961$	0.4050	0.0526	$F_{(119, 1785)} = 0.971$	0.5720	0.0608	$F_{(238, 1785)} = 0.7602$	0.9964	0.0921
Isolation 2	$F_{(5, 3570)} = 4.592$	0.0031	0.2045	$F_{(119, 3570)} = 1.991$	<0.0001	0.0622	$F_{(595, 3570)} = 0.7469$	1.000	0.1107

Table 1b. Results of two-way RM ANOVA analyzing the effects of the socialization episodes, the time of day, their interaction on time series of locomotor activity behavior of Single, Pair, and Group housed spotted munia. Effect size estimates showing the magnitude of response are presented as partial Eta squared (η_p^2). F and p values in bold indicate points of significant difference. For statistical significance, the alpha was set at 0.05.

	Two-way ANOVA statistics								
Housing condition	Socialization episodes (S1/S2/S3)			The time of day			Interaction (Socialization episodes x The time of day)		
	F value	p value	η_p^2	F value	p value	η_p^2	F value	p value	η_p^2
Single	$F_{(2, 1785)} = 13.80$	0.0004	0.2735	$F_{(119, 1785)} = 6.757$	<0.0001	0.3105	$F_{(238, 1785)} = 4.947$	<0.0001	0.3974
Pair	$F_{(2, 1785)} = 7.132$	<0.0001	0.2638	$F_{(119, 1785)} = 4.971$	0.0067	0.2489	$F_{(238, 1785)} = 6.947$	<0.0001	0.4808
Group	$F_{(2, 1785)} = 3.493$	0.0569	0.2039	$F_{(119, 1785)} = 6.163$	<0.0001	0.2913	$F_{(238, 1785)} = 5.297$	<0.0001	0.4139

Table 2a. Results of two-way RM ANOVA analyzing the effects of housing condition, the time of day, and their interaction on hourly distribution profile of rest and activity of spotted munia under different episodes of social interaction; Social 1, Social 2, Isolation 1, local 3, and Isolation 2. Effect size estimates showing the magnitude of response are presented as partial Eta squared (η_p^2). F and p values in bold indicate points of significant difference. For statistical significance, the alpha was set at 0.05.

Two-way ANOVA statistics									
The episode of social interaction	Housing condition			The time of day			Interaction (Housing condition x The time of day)		
	F value	p value	η_p^2	F value	p value	η_p^2	F value	p value	η_p^2
Social 1	$F_{(2, 1785)} = 0.29$	0.7545	0.0180	$F_{(119, 1785)} = 67.66$	<0.0001	0.9065	$F_{(238, 1785)} = 2.42$	<0.0001	0.1023
Social 2	$F_{(2, 1785)} = 0.52$	0.6022	0.1314	$F_{(119, 1785)} = 12.55$	<0.0001	0.7417	$F_{(238, 1785)} = 1.43$	<0.0001	0.1411
Isolation 1	$F_{(5, 3570)} = 20.82$	<0.0001	0.6296	$F_{(119, 3570)} = 1.77$	<0.0001	0.0579	$F_{(595, 3570)} = 1.30$	<0.0001	0.1853
Social 3	$F_{(2, 1785)} = 1.25$	0.3160	0.0948	$F_{(119, 1785)} = 2.30$	<0.0001	0.1662	$F_{(238, 1785)} = 0.83$	0.9682	0.0745
Isolation 2	$F_{(5, 3570)} = 6.82$	0.0002	0.4291	$F_{(119, 3570)} = 2.03$	<0.0001	0.0785	$F_{(595, 3570)} = 0.86$	0.9883	0.1076

Table 2b. Results of two-way RM ANOVA analyzing the effects of the socialization episodes, the time of day, their interaction on hourly distribution of rest-activity profile of Single, Pair, and Group housed spotted munia effect size estimates showing the magnitude of response are presented as partial Eta squared (η_p^2). F and p values in bold indicate points of significant difference. For statistical significance, the alpha was set at 0.05.

	Two-way ANOVA statistics								
Housing condition	Socialization episodes (S1/S2/S3)			The time of day			Interaction (Socialization episodes x The time of day)		
	F value	p value	η_p^2	F value	p value	η_p^2	F value	p value	η_p^2
Single	$F_{(2, 1785)} = 4.723$	0.0257	0.1083	$F_{(119, 1785)} = 15.22$	<0.0001	0.5034	$F_{(238, 1785)} = 5.114$	<0.0001	0.4053
Pair	$F_{(2, 1785)} = 0.5637$	0.5807	0.0345	$F_{(119, 1785)} = 7.214$	<0.0001	0.3247	$F_{(238, 1785)} = 3.636$	<0.0001	0.3265
Group	$F_{(2, 1785)} = 1.113$	0.3543	0.0705	$F_{(119, 1785)} = 14.75$	<0.0001	0.4958	$F_{(238, 1785)} = 5.153$	<0.0001	0.4073

Phase shift in activity onset. Like total activity and activity duration, phase shift in the onset of activity also remained unaffected by the housing conditions at S2 ($p > 0.05$; Phase shift; Social 2; in Table 3). It was indifferent among Single, Pair and Group housed birds.

Table 3. Results of one-way ANOVA analyzing the effects of each episode of social interaction on circadian variables related to locomotor activity behavior of Single, Pair, and Group housed spotted munia. Effect size estimates showing the magnitude of response are presented as Eta squared (η^2). F and *p* values in bold indicate points of significant difference. For statistical significance, the alpha was set at 0.05. Here at isolations, singles were compared to the Pair host and Group host.

	One-way ANOVA statistics			
Circadian variables	Episode of social interaction	$F_{(2, 15)}$ value	*p* value	η^2
Total activity counts	Social 1	1.845	0.1922	0.1974
	Social 2	1.639	0.2271	0.1793
	Isolation 1	0.5071	0.6162	0.0633
	Social 3	0.9607	0.4050	0.1135
	Isolation 2	1.409	0.2750	0.1581
Activity duration	Social 1	0.2841	0.7566	0.0365
	Social 2	0.5257	0.6016	0.0655
	Isolation 1	1.868	1.886	0.1994
	Social 3	1.243	0.3166	0.1422
	Isolation 2	0.7923	0.4709	0.0955
Phase shift	Social 1	1.567	0.2410	0.1728
	Social 2	1.904	0.1832	0.2025
	Isolation 1	**4.506**	**0.0293**	0.3753
	Social 3	1.943	0.1777	0.2057
	Isolation 2	**3.977**	**0.0411**	0.3465
Circadian period	Social 1	2.189	0.1465	0.2260
	Social 2	0.3706	0.6965	0.0471
	Isolation 1	0.8053	0.4654	0.0969
	Social 3	0.8099	0.4634	0.0975
	Isolation 2	2.131	0.1532	0.2213

Table 3a. Results of two-way RM ANOVA analyzing the effects of housing condition, the time of day, and their interaction on circadian variables related to locomotor activity behavior of spotted munia. Effect size estimates showing the magnitude of response are presented as partial Eta squared (η_p^2). F and *p* values in bold indicate points of significant difference. For statistical significance, the alpha was set at 0.05.

	Two-way ANOVA statistics								
	Housing condition			Episodes of social interaction			Interaction (Housing condition x Episodes of social interaction)		
Circadian variables	$F_{(2,60)}$ value	*p* value	η_p^2	$F_{(4,60)}$ value	*p* value	η_p^2	$F_{(8,60)}$ value	*p* value	η_p^2
Total activity counts	1.423	0.2761	0.2252	**27.09**	**<0.0001**	0.6436	1.332	0.2457	0.1508
Activity duration	0.5429	0.5920	0.1678	**10.94**	**<0.0001**	0.4218	**2.359**	**0.0281**	0.2393
Phase shift	2.47	0.1186	0.1267	**7.66**	**<0.0001**	0.3650	**2.85**	**0.0093**	0.1876
Circadian period	0.70	0.5100	0.0410	1.27	0.2900	0.0783	1.10	0.3768	0.1278

Table 3b. Results of one-way RM ANOVA analyzing the effects of socialization (Social 1, Social 2, and Social 3) on circadian variables related to locomotor activity behavior of Single, Pair, and Group housed spotted munia. Effect size estimates showing the magnitude of response are presented as Eta squared (η^2). F and *p* values in bold indicate points of significant difference. For statistical significance, the alpha was set at 0.05.

	One-way ANOVA statistics			
Circadian variables	Housing condition	$F_{(2, 10)}$ value	*p* value	η^2
Total activity counts	Single	**13.80**	**0.0013**	0.6480
	Pair	**8.709**	**0.0065**	0.4876
	Group	**7.724**	**0.0094**	0.3177
Activity duration	Single	**13.12**	**0.0016**	0.3868
	Pair	1.071	0.3790	0.0704
	Group	**6.239**	**0.0174**	0.1291
Phase shift	Single	0.4603	0.6438	0.2969
	Pair	1.608	0.2480	0.3098
	Group	1.703	0.2310	0.3115
Circadian period	Single	0.2854	0.7576	0.0259
	Pair	2.235	0.1577	0.2355
	Group	0.5482	0.5944	0.0489

Table 3c. Results of Student's *t*-test for Pair and one-way ANOVA for Group, analyzing the effects of Isolation 1 and 2 on circadian variables related to locomotor activity behavior between or among host and guest(s) in Pair and Group housed spotted munia, respectively. Effect size estimates showing the magnitude of response are presented as Glass's δ for Pair and Eta squared (η^2) for Group. F and *p* values in bold indicate points of significant difference. For statistical significance, the alpha was set at 0.05.

| | | PAIR | | GROUP | | |
| | | Student's t-test | | One-way ANOVA statistics | | |
Circadian variables	Episode of Isolation	p value	Glass's δ	$F_{(2, 15)}$ value	p value	η^2
Total activity counts	Isolation 1	**0.0001**	3.860917	**5.975**	**0.0123**	0.4435
	Isolation 2	**0.0072**	5.914252	2.644	0.1039	0.2607
Activity duration	Isolation 1	**<0.0001**	3.329111	**21.08**	**<0.0001**	0.7377
	Isolation 2	**0.0066**	1.648037	**5.233**	**0.0189**	0.4110
Phase shift	Isolation 1	**0.0074**	2.040875	0.3434	0.7148	0.1536
	Isolation 2	**0.0161**	1.714086	2.927	0.0845	0.2698
Circadian period	Isolation 1	0.9695	0.030148	0.5898	0.5668	0.0729
	Isolation 2	0.1024	0.955572	0.09427	0.9106	0.0124

Table 4. Results of one-way RM ANOVA analyzing the effects of social interaction protocol on body mass of Single, Pair, and Group housed spotted munia compared at three time-points during the experiment. Effect size estimates showing the magnitude of response are presented as Eta squared (η^2). F and *p* values in bold indicate points of significant difference. For statistical significance, the alpha was set at 0.05.

One-way ANOVA statistics				
Housing condition		$F_{(2, 10)}$ value	p value	η^2
Single		0.5096	0.6155	0.0383
Pair	Host	0.7592	0.4932	0.0772
	Guest	**8.478**	0.0070	0.3548
Group	Host	2.392	0.1416	0.1438
	Guest 1	**4.874**	0.0333	0.3685
	Guest 2	3.000	0.0954	0.0943

Circadian period. Circadian period also remained unaffected by the housing conditions at S1 ($p > 0.05$; Circadian period; Social 1; in Table 3). It was indifferent among Single, Pair and Group housed birds.

Effect of Isolation 1 (I1) episode on locomotor activity characteristics
Time series of locomotor activity. During I1, the locomotor activity pattern was significantly affected by the housing condition and the time of day ($p < 0.05$; I1 in Table 1a). The amplitude of the time series of activity varied significantly between Group host and Group guest 1 (Figure 3ciii and civ) and also between Group host and Group guest 2 (Figure 3ciii and cv).

Hourly distribution of rest-activity. A significant effect of housing condition, the time of day, and their interaction was found on the hourly distribution of rest and activity behavior at I1 ($p < 0.05$; I1 in Table 2a). During I1, the distribution profile of rest and activity varied significantly between Pair host and Pair guest (Figure 4biii and biv), Group host and Group guest 1 (Figure 4ciii and civ), and also between Group host and Group

guest 2 (Figure 4ciii and cv). At I1, the rest and activity distribution profiles of hosts of Single, Pair, and Group were not different from each other (Figure 4aiii, biii, and ciii).

Total activity counts. Total activity counts were not affected by housing conditions at I1 ($p > 0.05$; Total activity counts; Isolation 1; in Table 3). It was indifferent among Single, Pair host, and Group host birds.

Activity duration. Activity duration also remained unaffected by the housing conditions at I1 ($p > 0.05$; Activity duration; Isolation 1; in Table 3). Like total activity, it was also indifferent among Single, Pair host, and Group host birds.

Phase shift in activity onset. Unlike total activity and activity duration, phase shift in the onset of activity got affected by the housing conditions at I1 ($p < 0.05$; Phase shift; Isolation 1; in Table 3). It was significantly different between Pair host and Group host. It could be seen in Figure 5cii and 5ciii for Pair host and Group host respectively.

Circadian period. Circadian period remained unaffected by the housing conditions at I1 ($p > 0.05$; Circadian period; Isolation 1; in Table 3). It was indifferent among Single, Pair host, and Group host birds.

Effect of Social 3 (S3) episode on locomotor activity characteristics

Time series of locomotor activity. During S3, locomotor activity pattern was neither affected by the time of the day not affected by the housing condition. The interaction of these two factors also remained ineffective ($p > 0.05$; S3 in Table 1a).

Hourly distribution of rest-activity. Unlike S1 and S2, at S3, the hourly distribution of rest and activity profile was significantly affected by the time of day only and not by the housing condition or their interaction ($p < 0.05$; S3 in Table 2a).

Total activity counts. Like S1 and S2, the total activity counts were not affected by housing conditions at S3 also ($p > 0.05$; Total activity counts; Social 3; in Table 3). It was indifferent among Single, Pair, and Group housed birds.

Activity duration. Activity duration remain unaffected by the housing conditions at S3 also like S1 and S2 ($p > 0.05$; Activity duration; Social 3; in Table 3). Like total activity, it was also indifferent among Single, Pair, and Group housed birds.

Phase shift in activity onset. Like total activity and activity duration, phase shift in the onset of activity also remained unaffected by the housing conditions at S3 ($p > 0.05$; Phase shift; Social 3; in Table 3). It was indifferent among Single, Pair, and Group housed birds.

Circadian period. At S3 also, circadian period followed the same course as S1 and S2, and remained unaffected by the housing conditions ($p > 0.05$; Circadian period; Social 3; in Table 3). It was indifferent among Single, Pair, and Group housed birds.

Effect of Isolation 2 (I2) episode on locomotor activity characteristics

Time series of locomotor activity. Like I1, during I2 also, we found a significant effect of housing condition and the time of the day on locomotor activity pattern ($p < 0.05$; I2 in Table 1a). The pattern of activity varied significantly between the Pair host and Pair guest (Figure 3bvi and bvii).

Hourly distribution of rest-activity. During I2, we found a significant effect of housing condition and the time of day on rest and activity distribution profile ($p < 0.05$; I2 in Table 2a). During I2, the distribution profile of rest and activity profile varied between the Pair host and Pair guest (Figure 4bvi and bvii). Again, at I2 also the rest and activity distribution profiles of hosts of Single, Pair, and Group were not different from each other (Figure 4av, and cvii).

Total activity counts. Total activity counts were not affected by housing conditions at I2 like I1 ($p > 0.05$; Total activity counts; Isolation 2; in Table 3). It was indifferent among Single, Pair host, and Group host birds.

Activity duration. Activity duration also remained unaffected by the housing conditions at I2 too ($p > 0.05$; Activity duration; Isolation 2; in Table 3). Like total activity, it was also indifferent among Single, Pair host, and Group host birds.

Phase shift in activity onset. Again, like I1, at I2 also the phase shift in the onset of activity got affected by the housing conditions ($p < 0.05$; Phase shift; Isolation 2; in Table 3). It was significantly different between Single bird and Pair host (Figure 5ci and cii) and also between Single bird and Group host (Figure 5ci and c3).

Circadian period. Circadian period remained unaffected by the housing conditions at I2 also ($p > 0.05$; Circadian period; Isolation 2; in Table 3). It was indifferent among Single, Pair host, and Group host birds.

Section II: effect of social housing (comparing S1, S2 and S3 at a particular housing condition)

Time series of locomotor activity

We found that the locomotor activity pattern was significantly affected by the socialization episodes, time of the day, and their interaction in Single and Pair housed birds ($p < 0.05$; Single and Pair; Table 1b). While in Group, the locomotor activity pattern was significantly affected by the time of day and its interaction with the socialization episodes only ($p < 0.05$; Group; Table 1b). In Single, Pair as well as Group, the locomotor activity pattern was significantly different between S1 and S2 and also between S1 and S3 but not between S2 and S3 (Single: Figure 3ai, and aiv; Pair: Figure 3bi, and bv; Group: Figure 3ci, and cvi).

Hourly distribution of rest-activity

We found that per hour rest-activity distribution was significantly affected by the socialization episodes, time of the day, and their interaction in Single housed birds ($p < 0.05$; Single; Table 2b). It was significantly different among S1, S2 and S3 (Figure 4ai, and aiv). While in Pair and in Group, the per hour rest-activity distribution was significantly affected by the time of the day and its interaction with the socialization episodes ($p < 0.05$; Pair and Group; Table 2b). In Pair, it was significantly different between S1 and S2 (Figure 4bi-4) while in Group, it was significantly different among all the three episodes of socializations (Figure 4ci, and cvi).

Effect on total counts

We found that total counts were significantly different in all the housing conditions ($p < 0.05$; Table 3b). In Single, total counts significantly decreased in S2 and S3 relative to S1 (Figure 5ai), while in Pair total counts decreased significantly in S2 relative to S1 (Figure 5aii), and in Group, the total activity counts decreased significantly in S2 and S3 as compared to S1 (Figure 5aiii).

Effect on activity duration

Activity duration varied significantly in Single housing and Group housing conditions only when compared among S1, S2 and S3 ($p < 0.05$; Table 3b). In Single, activity duration/day significantly decreased in S2 and S3 relative to S1 (Figure 5bi), while in Group the activity duration decreased significantly in S2 relative to S1 and S3 (Figure 5biii), and in Pair, the activity duration remained unaltered among the episodes of socialization (Figure 5bii).

Effect on phase shift

To study the effect of S1, S2, and S3 on daily phase shift, we compared it amongst these episodes in a specific housing condition and found that phase shift did not vary significantly in any of the housing conditions ($p > 0.05$; Table 3b). We also found that the number of birds could be distinguished according to whether they tended to advance or delay their phase of activity onset in different episodes of social interaction as summarized in Supplementary Table S1. Based on the frequency under constant light conditions, it is shown that during socializations birds tended to advance their phase of onset of activity.

Effect on circadian period

Circadian periodicity was compared among S1, S2 and S3 in a specific housing condition and found that circadian periodicity did not vary significantly in any of the housing conditions ($p > 0.05$; Table 3b). The Chi-square periodograms of Single, Pair, and Group housed birds are shown in Supplementary Figures S2–6 for each episode. Additionally, we are showing that in terms of circadian periodicity, the clock can be categorized as fast, slow, and equal to 24 h. On this basis, we calculated the frequency distribution of birds under constant light conditions; as those in which the period of daily activity cycle is less than 24 h (fast clock), more than 24 h (slow clock), and where the calculated periodicity is equal to 24 h. The frequency distribution is summarized in Supplementary Table S2. We found that during socialization clock tended to run slow with a >24 h periodicity.

Section III: effect of isolations on the locomotor activity of Single/host birds and the guest birds post-socialization

Time series of locomotor activity

The activity pattern of Single bird, Pair host, and Group host was not different from each other at I1 as well as at I2 (Figure 3aiii, and ciii and av, bvi and cvii, respectively; stats shown in supplementary data).

Hourly distribution of rest-activity

The hourly distribution of rest-activity profile was significantly affected by the time of day as well as its interaction with the housing condition at I1 among Single bird, Pair host, and Group host (Figure 4aiii, biii and ciii; stats shown in supplementary data). It was not affected by the housing conditions. Bonferroni posttest did not show a difference between the housing conditions. At I2 however, the hourly rest-activity distribution profile was not different among Single, Pair host and Group host (Figure 4av, bvi and cvii; stats shown in supplementary data).

Effect on total counts

At I1 and I2 in Pair, the total counts of the guest were more than that of the host ($p < 0.05$; Table 3c; Figure 5aii). While in Group, the total counts were significantly different at I1 only ($p < 0.05$; Table 3c) where the total counts were higher in guest 1 than the host in Group (Figure 5aiii).

Effect on activity duration

In Pair, the guest birds were active for a longer duration than the host at both I1 and I2 ($p < 0.05$; Table 3c; Figure 5bii). Like Pair, in Group also, the activity duration was significantly different at both I1 and I2 ($p < 0.05$; Table

3c). Daily activity duration was higher in guest 1 and guest 2 than in the host bird at both I1 and I2 (Figure 5biii).

Effect on phase shift

At I1 and I2 in Pair, the host significantly delayed its phase of onset of activity, whereas the guest advanced ($p < 0.05$; Table 3c; Figure 5cii). While in Group, the phase shift in the onset of activity remained unaffected at both I1 and I2 ($p > 0.05$; Table 3c; Figure 5ciii). Based on the frequency of birds under constant light conditions, Supplementary Table S1 shows that during isolations birds were inclined to delay their phase of onset of activity.

Effect on circadian period

It was found that circadian periodicity did not get affect by both I1 and I2 in Pair ($p > 0.05$; Table 3c). Also, among the Group members, there was no difference in the tau at both I1 and I2 ($p > 0.05$; Table 3c). Supplementary Table S2 shows that during isolation the endogenous clock tended to run faster with a periodicity of <24 h.

Effect on body mass

Body mass was recorded to study the effect of social interaction protocol on spotted munia. Body mass was compared among the three time-points, namely, LD_1, LD_2, and LL_{dim} in each housing condition, and we found that body mass did not vary significantly in Single birds ($p > 0.05$; Table 4; Figure 6a). In Pair, the body mass of the host remained unaffected ($p > 0.05$; Table 4; Figure 6b) but it differed significantly for the Pair guest ($p < 0.05$; Table 4). The Pair guest's body mass decreased at LL_{dim} time-point relative to LD_1 and LD_2 time-points (Figure 6c). Similarly, in Group, the body mass of the host and guest 2 did not change upon social interaction ($p > 0.05$; Table 4; Figure 6d and f), but it got significantly changed for guest 1 ($p < 0.05$; Table 4). The body mass of Group guest 1 decreased significantly at the LL_{dim} time-point compared to LD_2 time-point (Figure 6e), whereas for Group guest 2, the body mass remained unchanged (Figure 6f).

Discussion

Behavior reflects the physiological well-being of an organism and therefore, locomotor activity pattern, pictorially represented as an actogram imitates the biological status of the body. Under the S1 episode, a combination of light and social cues operated synergistically; empowering synchronization of the locomotor

activity rhythm with the surrounding, rather than social cues alone (Favreau-Peigne et al. 2009); therefore, initially, we kept the birds under LD cycle as Single, Pair, and Group. This synchronization during constant light conditions was feasible, since in the biological system, circadian rhythms are persisting, endogenously generated oscillations with a periodicity of about 24 h and continue when regular time cues are lacking (Davidson and Menaker 2003). However, upon isolations, the birds showed drastic variation in their behavior and physiology as discussed ahead in the paper. These birds live in flocks during the non-breeding season (Ali and Ripley 1999). It could be expected that social isolation during the non-breeding season would impact the behavior, but Single housed birds taken from the same set of captured birds did not show arrhythmicity and free ran absolutely normally, thereby suggesting that it was the influence of housing condition and social interaction that affected the activity behavior.

Variation in the pattern of time series of locomotor activity

Subjecting birds to social interactions affected their daily behavior by disrupting the activity pattern and it is obvious that for increased activity the birds must have made some temporal adjustments. Time series (Figure 3) is providing evidence for alteration in daily activity profiles of Single, Pair, and Group housed birds across the span of five days. At S1, the pattern varied possibly because the number of individuals in Pair and Group was more relative to Single; Group had three individuals which was still more than Pair that had two. Also, since Pair and Group had light as well as social cues to synchronize with, their activity enhanced further because of the company in a well illuminated ambience and its pattern got varied, but the enhancement in the activity of Pair was at par with the Single birds. Similarly, during S2 also the birds under different housing conditions followed the same trend but with a dampened amplitude, suggesting that LL_{dim} constant light condition in general reduced the locomotor activity qualitatively as well as quantitatively, and any variations obtained were owing to the influence of social cues. During I1, Group guests were more active probably owing to the effect of social isolation-induced loneliness upon separation from the cohabitants.

According to Hawkley and Capitanio (2015), loneliness is inaccessible in non-human animals; however, isolation-induced physiological changes in social animals reflect those experienced by humans encountering loneliness. If we look at the studies on birds; Aydinonat et al. (2014), have revealed in African grey parrots that

telomere length becomes significantly shorter in socially isolated birds as compared to Pair housed birds. Interestingly, short telomeres are known to be linked with a higher risk of various types of age-related diseases in human beings (Sanders and Newman 2013). Likewise, European starlings when subjected to separation from their group members, showed increased activity and corticosterone concentration (Apfelbeck and Raess 2008). There are several other accounts for a direct relationship between corticosterone level and locomotor activity in birds (Belthoff and Dufty 1998; Wingfield et al. 1998). JM. George et al. (2020) demonstrated that even acute social isolation can affect gene expression and DNA methylation in forebrain areas associated with higher cognitive functions in zebra finches, and concluded that social isolation elicits epigenetic mechanisms that might affect the processing of ongoing experience; loneliness for the sake. Another factor could be the introduction of a novel environment (cage change); though the cages were identical in terms of dimensions. Singh et al. (2010) reported that buntings isolates decreased the nighttime activity possibly due to cage change.

At S3, however, all the housing conditions had similar diel activity patterns, which seemed to resume a normal course after isolation-induced disruptions. But during I2 in Pair, the host and guest differed from each other in their pattern of activity, perhaps because the guest seemed to revert to a normal diel cycle after pulsatile social interactions, to maintain homeostasis. Adjustments in circadian rhythm profoundly affect the physical and psychological homeostasis of an individual (Atcheson and Tyler 1975), and elements of the physiological defense mechanisms; the mediators, are secreted in response to disturbed homeostasis to restore it, and therefore, serve as an important link against stress during primary defense (Munck et al. 1984). Time series of locomotor activity was affected by episodes of socialization in Single and Pair housed birds simply due to change in light regimen and intermittent episode of isolation respectively. In Group though, the time of day factor dominated over socialization. While at isolations, Single bird's activity pattern resembled to that of Pair host and Group host with no significant differences.

Differential rest and activity distribution profile

The rest and activity distribution profiles were mirror images of each other as seen in Figure 4. During S1, rest and activity distribution profiles were synchronized with the light and dark cycle whereas, under LL_{dim}, the birds were active according to their subjective day and

subjective night. "Subjective day" is the time during which the animal remains awake and performs its daily activity under the influence of the biological clock, and "subjective night" is the clock-controlled time during which the animal takes rest. At S2 also, the daily pattern of rest and activity was sustained throughout the day like S1, possibly because birds were well synchronized in Pair and Group and well entrained in Single housed condition. Upon I1, the distribution profile of rest and activity in guest(s) relative to host varied, and there was an indistinct demarcation between subjective day and night in the activity of guests. This might have happened because the guest birds were longing for their cage mates in a naïve surrounding, and got unrest. Besides, we strongly believe that the novelty of the environment contributed to this behavior too. We also consider that isolation situated birds under stress (Hennessy 1997) to a certain level, maybe through underlying physiological and psychological disruption (Onaka et al. 2012), since, under constant dim light conditions, they had only social cues to synchronize with.

While at S3, again we saw the evidence of coherence among the members of a cohoused assembly, because now again they have their companion back, with whom they were initially housed; and that resulted in the loss of restlessness. While at I2, Pair guest which probably headed towards a state of homeostasis, acquired distinguished activity during subjective day and night in contrast to its host and acquired rhythmic pattern. Hourly distribution profile of rest-activity indicated that Single housed birds significantly changed their activity counts/h from S1 to S3 through S2 mainly due to change in light regimen and duration of the experiment under free-running condition. In Pair and Group, this comparison was not directly affected by socialization episode but by the interaction of socialization with the time of day. While at I1, Single, Pair host, and Group host had differences in rest-activity distribution profile activity, this was not found at I2. This suggests that since I1 occurred first time in the experiment, it made birds more unrest than I2. It also indicates that I1 was more difficult for birds compared to I2.

Account for the total counts in a day

To seek a possible approach that might have been controlling such behavioral expression, we analyzed variables associated with circadian rhythms by excavating data from the activity profile. Subsequently, we found that total activity counts were highest in S1 in all the housing conditions compared to S2 and S3 because the

light cue being the strongest *Zeitgeber* provided birds with an appropriate ambiance to perform their daily exploratory behavior more rigorously than the constant light condition. For increased total activity counts during isolations in guests of both Pair and Group, we consider that two factors worked upon; loss of companion and change of cage. Companion might have served as a strong cue for social entrainment resulting in strong synchronization of activity behavior during socialization, and isolating them might have resulted in loss of this synchrony leading to psychosocial constrain and restlessness which might have played a role in intensifying the total counts. Again, we attribute the role of novelty of the environment too; by the change of cage, for the intensification of the activity.

Although all the birds were in isolation at the end, the guest of Pair was more active than the host. This happened probably because following separation, the Pair guest was seeking its companion as they were socialized with each other, and also because the guest's environment changed in terms of place. Host birds, on the other hand, were kept back in the same cage, so they could be refrained from stress (if any) due to the novel environment. This was prominent in Pair as they had only one guest to synchronize with and the idea of socialization was a rewarding situation for them. The likelihood of handling stress did not seem to work here as all the birds were handled at the time of cage change, including Single birds. So, this hyperactivity was most likely motivation-driven (each Group member had two guests, therefore, it might have been difficult to match the rhythm with any one of them, so, this motivation-driven hyperactivity was not seen in Group at I2; as seen for Pair). A study on mice (Van Loo et al. 2001) suggested that the mice were motivated for social contact with familiar mice; irrespective of the fact whether they were the dominant or the subordinate one. Also, Panksepp and Lahvis (2007), have reported in their study that most mouse strains they used, associated environmental signals related to conspecifics' presence as a more positive affective state and considered it as a rewarding situation.

Alteration in the activity duration might have played the mechanistic role

Daily activity duration is a very crucial gear associated with the body's homeostasis, so we investigated the daily activity duration of birds. The activity duration was higher during S1 (most probably because of LD cycles) relative to S2 and S3 in Single and Group birds only and not in the Pair. This suggests that light and social cues together synchronized the members of Pair stronger

than that of Group; therefore, their activity duration was almost equal in S1, S2, and S3. Isolation increased the activity duration of guest(s) relative to host in Pair and Group obviously by reducing the duration of rest and affecting the physiology of sleep; which perhaps was enhanced by shifting to a novel environment. Similar results have been found in a study on mice where it is suggested that the social context of isolation stress may adversely affect the quantity and quality of sleep (Kaushal et al. 2012). On the other hand, during socializations, the birds were active for almost half of the day and took rest for the other half which comprises a normal activity-rest cycle for a diurnal animal. During socialization, the birds were active together and resting together, as they would have been in the presence of a light/dark cycle; but for the amplitude, thereby letting us ponder that there was an existence of mutual synchronization between/among them. Here, by the term "mutual synchronization", we mean that cohoused birds were active together or resting together, as revealed by the consolidated bouts of their activity or rest. So, we consider that activity duration/day played a mechanistic role in regulating birds' behavior under the influence of social cues.

Daily adjustment defined the extent of dependency

The internal circadian clocks are used to program the physiological and behavioral activity within the 24 h time domain. During the free run, circadian periods vary slightly from 24 h (Davidson and Menaker 2003), characterized by a period that is close to, but not exactly, 24 h in length, which possibly contributed to the phase shift in the onset of activity in our birds. In our study, we did not find any phase shift in the onset of activity in birds during S1, S2, and S3, which suggests that there was a strong social cohesion between members of a cohoused assembly when kept together. Other than this, Single birds also did not show any phase shift in the activity onset from one episode to the other, possibly because they were simply free-running under a constant light condition and did not experience either social cues or social isolation-induced loneliness sort of thing. For group living, unity among the members of a Group is a criterion, and synchronization of activity behavior is considered to be fundamental for social cohesion. According to Gautrais et al. (2007), the extent of synchrony within a group is accredited to the mutual adjustment of behavior by group members. Subjecting birds to socialization followed by isolation resulted in a significant change in the phase of activity onset. In the case of Pair but not in the Group, the delaying of the phase of activity onset of host relative to guest at I1, as

well as I2, indicates that there was stronger synchrony between the Pair members than Group, as isolating them led to phase shift perhaps due to loneliness and dependency on the partner. Also, in hamsters, it has been found that cage change contributes to phase shifting (Mrosovsky 1988). On the other hand, Group members followed their obvious diel pattern of activity (Figure 3cvii–3); hence, no phase shift in the onset of activity was observed. Studies on *Drosophila* kept in groups in constant settings displayed a more coherent phase in their circadian activity-rest rhythm compared to flies upheld individually, suggesting that the clock-controlled locomotor activity rhythm in flies is altered by clocks of cohabitants possibly through social interactions (Krupp et al. 2008; Levine et al. 2002).

The robustness of the clock prevented change in periodicity

After socially isolating the birds, we found that quantitatively and qualitatively, the rest and activity distribution and onset of activity changed, but because of the endogenous origin of the circadian periodicity, it remained close to 24 h and did not show any significant change throughout the experiment (Supplementary Figures S2–6). This affirms the robustness of the circadian clock in terms of periodicity, thereby, indicating that social interaction reinforced cohesion among members of a housing condition without significantly altering the endogenous period of the rhythm. This may be advantageous for the animal to combat harsh climatic conditions by huddling, predation, and increasing the chances of breeding, etc. on a temporal basis. Therefore, we consider that a constant light environment enriched with social cues convinced the birds in a cohoused assembly perhaps; firstly, to match their temporal activities for energy conservation (e.g., by huddling) which subsequently offers a considerable thermoregulatory/metabolic reserve (Yates et al. 1991), and secondly, to temporally distribute different activities at a specified time of the day by taking time cues from each other to maintain a state of synchrony and homeostasis.

Decrease in body mass unveiled the negative aspect of social isolation

Supporting this behavioral data, as physiological indices we recorded the body mass. A decrease in body mass of the guest bird in Pair as well as in Group indicates that body mass was influenced by pulsatile social interaction, and loss of companion negatively affected the body mass. Similar results have been found in a study on mice (van Leeuwen et al. 1997). Concurrent

hyperactivity (Figure 3) for a longer duration (Figure 5), and consequent rest for a shorter period by guest birds (Figure 4), probably resulted in a decline in their body mass (Figure 6). The outcomes obtained could also be due to the cumulative effect of constrain of being in a constant light environment, handling stress and effect of cage change; accompanied by social isolation. This could be affirmed by no change in body mass of Single housed birds.

Also, the body mass of hosts remained unaffected because, unlike guests, they did not show hyperactivity in their caged environment. Social interaction is linked to neuronal circuitry governing sleep (Beattie et al. 2015); subsequently, its impact on the physiological state of the body, considering alteration in body mass as a marker, was very much likely in our study outcome. We recorded body mass to assess the responsivity of the birds to pulsatile social interactions and found that pulsatile social isolation induced a reduction in body mass thereby affirming the constrains put on by social isolation and cage changing on the birds' behavior and physiology. Apfelbeck and Raess (2008) have shown in their study on European starlings that social separation from members of a group resulted in reduced body mass.

Conclusion

We demonstrated that social cues can modulate the circadian characteristics in spotted munia and that social isolation negatively affected their activity behavior and physiology. Outcomes from the study suggest that if social animals are subjected to enforced isolation, they show similar behavior as mammals during loneliness. Birds acquired a mechanistic approach to attain homeostasis by altering the pattern, intensity, duration, and phase relation of their locomotor activity. We propose that such pulsatile social contact and social isolation simulated a social jetlag-like situation in birds where they get subjected to undesirable physiological constraints resulting in phase shift and alteration in the rest and activity distribution pattern to which novel environment possibly has contributed to. We also consider that the role of social cues in studies based on animals is not just confined to synchronizing or desynchronizing the biological clock to the given stimuli but also to better comprehend the costs of phase shift, social isolation-induced loneliness, and other related physiological consequences such as a decline in body mass. We addressed the impact of socialization and isolation by behavioral observations, but the data interpretation largely depended on presuming the physiological aspects.

Thus, this study will help in designing future experiments that can address the physiological regulation and downstream events governing such behavioral expressions in a social bird, and opening new avenues to address how heterospecific species respond to pulsatile social interactions.

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Data availability statement

The data that support the findings of this study are available at figshare (https://doi.org/10.6084/m9.figshare.23295800.v3) and further can be asked for from the corresponding author, [SR], upon reasonable request.

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