Circadian rhythm determines the timing of activity, and ingestive and grooming behaviours in Indian house crows, *Corvus splendens*

S. K. Tahajjul Taufique, Neelu Anand Jha and Vinod Kumar*

Department of Zoology, University of Delhi, Delhi 110 007, India

Corvids show an episodic memory with the temporal information on 'when' an event has actually occurred. We propose that crows keep the time and duration of an event by involving the endogenous circadian clocks, reliably measured in behavioural rhythms. The aim of the present study is to find out if circadian rhythms were involved in the organization of daily behavioural patterns in Indian house crows, *Corvus splendens*. We monitored the activity, ingestive (feeding: eating and drinking) and grooming (preening) behaviours in crows sequentially exposed to 12 h light : 12 h darkness (12 L : 12 D) and dim constant light (LL$_{dim}$) for one and three weeks respectively. The behavioural patterns were synchronized and diurnal was under 12 L : 12 D, while freeran had a circadian period under the LL$_{dim}$. In general, eating and drinking, not grooming, patterns were more closely related to the activity – rest cycle. These results show the persistence of circadian rhythms in behavioural patterns, and extend the idea that endogenous circadian clocks determine daily timing of the physiology and behaviour in Indian house crows, in particular, and birds, in general.

Keywords: Activity, behaviour, bird, circadian rhythms, crow, synchronization.

EVERY day, organisms show temporally segmented patterns in their physiology and behaviour, as an adaptation to maximize fitness in the prevailing periodic environment that they inhabit. Diurnal species are active during the day and inactive at night; the converse is true for nocturnals, which are active at night and inactive during the day. Thus, daily behavioural patterns can serve useful indices of the outcome of individuals' interaction with the prevailing abiotic (light, temperature) and biotic (food availability, conspecifics) components of the environment[1,2].

It is established that circadian timing is involved in the regulation of a wide array of daily functions, including daily cycles in activity–rest, sleep–wake and metabolism[3–5]. This is particularly noticed under constant environment of light or darkness (LL or DD), in which an individual exhibits the behavioural patterns (free runs) with its internal time[3,6,7]. In the natural 24 h environment,

however, organisms exhibit daily rhythms with the internal time synchronized with external periodicities, e.g. light-dark period (LD)[8–11].

Birds like other species show circadian rhythms in the regulation of their physiology and behaviour[8,9]. They are however a very heterogeneous group and exhibit significant species differences in the clock regulated functions. For example, the removal of pineal gland, which is a self-sustained circadian oscillator and regulates circadian functions, disrupts circadian rhythms in locomotor activity in several but not all songbirds, and has no effect on pigeon (*Columba livia*), Japanese quail (*Coturnix c. japonica*) and chicken (*Gallus domesticus*)[10].

In recent years, corvids has emerged as an important avian model system, particularly in studies aimed at unravelling processes underlying higher brain functions, viz. cognition. These birds show an episodic memory[12], with the temporal information on 'when' an event has actually occurred. Hence, they seem to have the sense of time and duration of a biological event[10–12]. Previous studies on corvids have shown a diurnal pattern with a morning peak in foraging[13] and predation, like nest raiding of other birds[14]. However, these field studies monitored only relative number of crows that foraged or the number of attempts made for predation. By measuring a number of behavioural patterns, we proposed to test whether crows keep the time and duration of an event by involving the endogenous circadian clocks. This has not been studied before in an experimental condition. The present study examined the patterns of activity–rest, ingestive and grooming behaviours in Indian house crows (*Corvus splendens*) sequentially exposed to LD and LL$_{dim}$ conditions.

Indian house crows (*n* = 6) were caught and individually housed in cages (size = 100 × 80 × 80 cm), each fitted with two perches and mounted with a passive infrared motion sensor (DSC, LC100 PI digital PIR detector, Canada). After acclimation, crows were exposed first to 12 h light : 12 h darkness (12 L : 12 D; L = 100 Lux, D = <1 Lux) for a week and then released into dim constant light (LL$_{dim}$; < 1 Lux) for about 3 weeks. Light was provided by compact fluorescent lamps (Phillips CFL lamp, 5W, 220–240 V). Food and water were provided *ad libitum*.

The IR sensor continuously detected the general movement (activity) of each crow and transmitted through separate channels to a computerized data acquisition system where it was stored and later analysed using the 'The Chronobiology Kit' software program, developed by Stanford Software Systems, USA. These records (actograms) were graphed, double-plotted and presented for each crow such that each successive day record was plotted sideways and underneath. In both LD and LL$_{dim}$ condition, a 7-day activity segment was selected and used for calculation of total and hourly distribution over 24 h. Similarly, the perch-hop, ingestive (feeding; eating and drinking) and grooming (preening) activity of each crow

were recorded by night-vision cameras (Axis 221 Network Camera, Axis communication, Sweden) consecutively over a two-day period in both LD and LL_{dim} condition. Each videograph was carefully observed and marked for each behaviour and analysed using the Observer XT 10 software (Noldus Information Technology, Wageningen, The Netherlands). Total and hourly distribution of activity over 24 h was calculated for all the four behavioural patterns.

Two-way analysis of variance (2-way ANOVA) analysed the effects of the time-of-day and light conditions. Bonferroni post-hoc test compared the values, if ANOVA indicated a significant difference. We used t-test for comparing the two groups. These statistical analyses were performed by GraphPad prism software program version 5.0 (San Diego, USA). Further, Rayleigh's test of uniformity was used to determine the distribution of each behavioural pattern and this was followed by Watson's U^2 test which determined differences between LD and LL_{dim} conditions[14]. These two tests were performed by Oriana 4.02 test (http://www.kovcomp.com/). $P < 0.05$ was considered as the significant difference.

In general, crows exhibited diurnal pattern in activity with consolidated bouts during daytime in 12 L : 12 D or subjective day in the LL_{dim} (Figure 1 b, c). Activity–rest pattern was thus synchronized with 12 L : 12 D and free ran under LL_{dim} with a circadian period (τ) of 24.4 ± 0.2 h (Figure 1 a). There was a significant effect of the light condition, time-of-day and interaction between the two on the general activity pattern (light condition: $F_{1,230} = 10.96$, $p = 0.0079$; time-of-day: $F_{23,230} = 22.09$, $p < 0.0001$; condition × time interaction: $F_{23,230} = 7.05$, $p < 0.0001$; 2-way ANOVA). However, total daily activity was significantly reduced under LL_{dim} than under the 12 L : 12 D ($t_{10} = 3.317$, $p = 0.0078$; t-test, Figure 1 d). Similar diurnal pattern was found in perch-hop activity (Figure 1 e, f), with a significant effect of the time-of-day and light condition × time-of-day interaction, but not of the light condition *per se* (light condition: $F_{1,230} = 2.545$, $p = 0.1418$; time-of-day: $F_{23,230} = 2.448$, $p = 0.0004$; condition × time-of-day interaction, $F_{23,230} = 1.791$, $p < 0.0171$; 2-way ANOVA). There was also significant reduction in daily total perch-hops under LL_{dim} than under the 12 L : 12 D ($t_{10} = 3.053$, $p = 0.0122$; t-test, Figure 1 g).
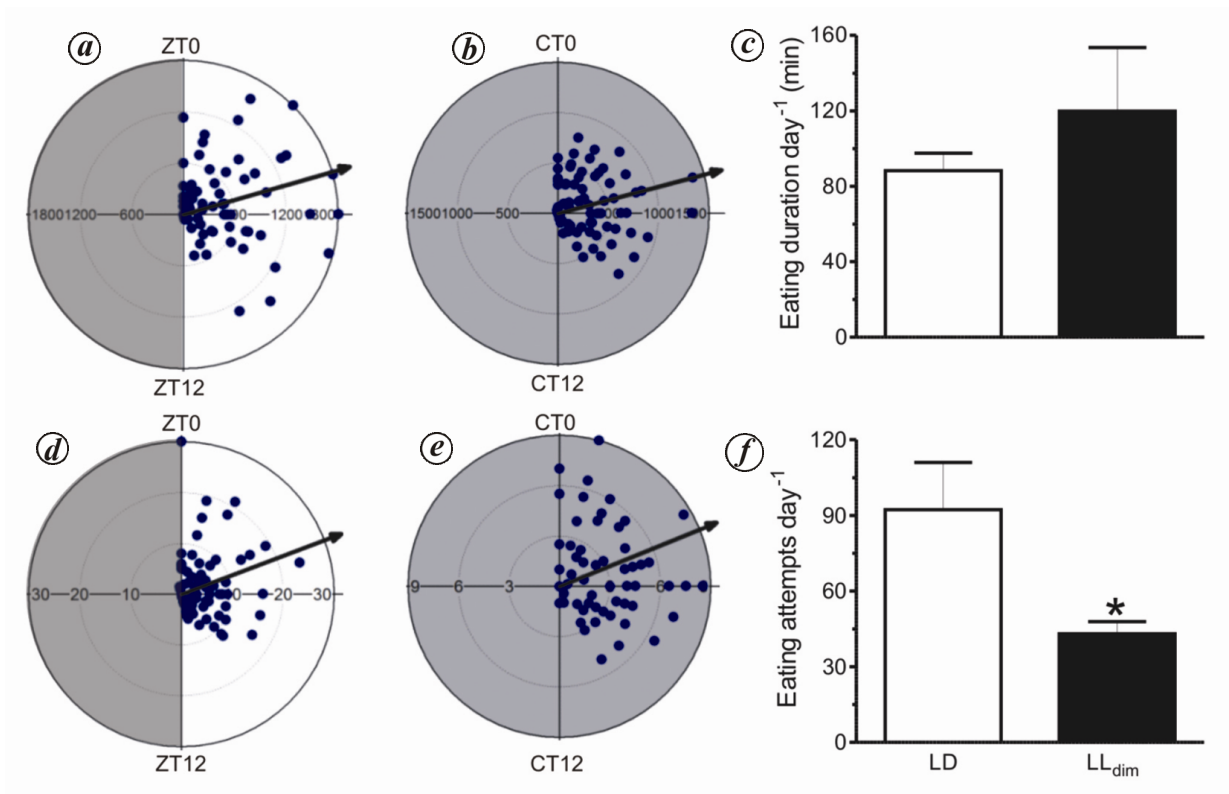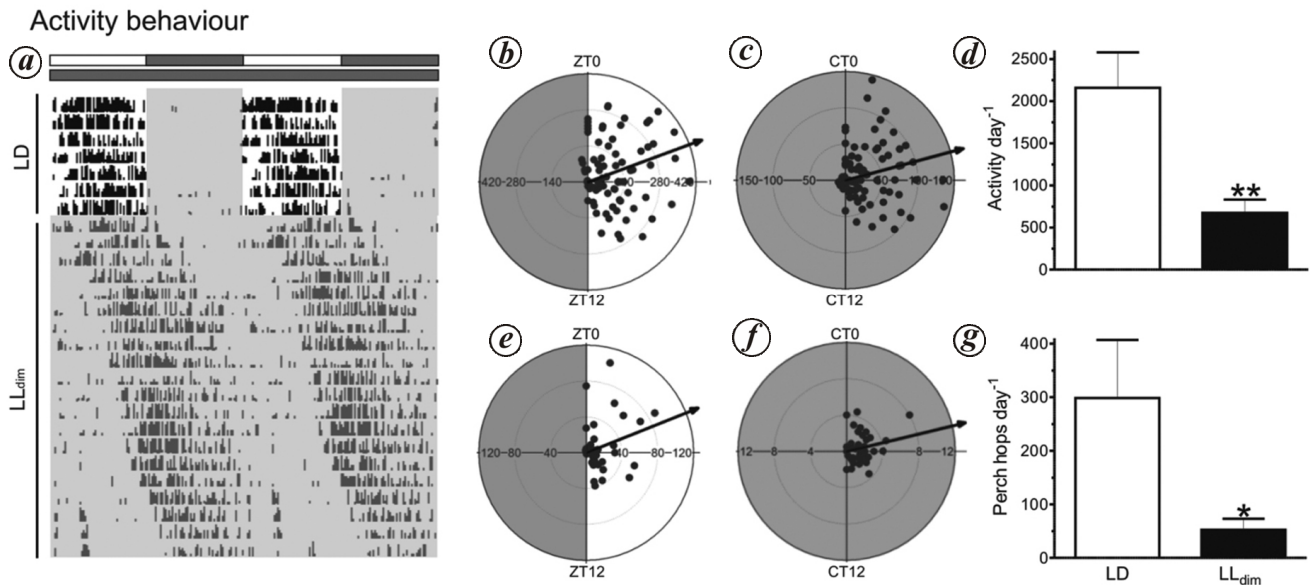
Eating and drinking behaviours were also diurnal in pattern, distributions during the day and subjective day in LD and LL_{dim} conditions respectively. Both, hourly feeding duration (Figures 2 a, b; 3 a, b) and attempts (Figures 2 d, e; 3 d, e) were mainly restricted during the day (or subjective day). There was a significant effect of the time-of-day, but not of the light condition or interaction between the two (eating only) on hourly eating (light condition: $F_{1,230} = 0.8167$, $p = 0.3874$; time-of-day: $F_{23,230} = 17.39$, $p < 0.0001$; condition × interaction: $F_{23,230} = 0.9015$, $p = 0.5967$; 2-way ANOVA) and drinking (light condition: $F_{1,230} = 2.665$, $p = 0.1336$; time-of-

day: $F_{23,230} = 6.783$, $p < 0.0001$; condition × time-of-day interaction: $F_{23,230} = 1.671$, $p = 0.0316$; 2-way ANOVA) patterns. On the other hand, there were significant effects of the light condition and time-of-day as well as the condition × time-of-day interaction on hourly attempts of eating (light condition: $F_{1,230} = 6.483$, $p = 0.0290$; time-of-day: $F_{23,230} = 13.66$, $p < 0.0001$; condition × time-of-day interaction: $F_{23,230} = 1.913$, $p = 0.0090$; 2-way ANOVA) and drinking (light condition: $F_{1,230} = 5.165$, $p = 0.0464$; time-of-day: $F_{23,230} = 8.145$, $p < 0.0001$; condition × time-of-day interaction: $F_{23,230} = 1.905$, $p = 0.0093$; 2-way ANOVA) patterns. However, there was no significant difference between LD and LL_{dim} conditions in the total daily duration of both eating and drinking (Figure 2 c, d; 3 f). At the same time, total daily attempts of eating, not drinking, was significantly higher under 12 L : 12 D than under the LL_{dim} condition ($t_{10} = 3.053$, $p = 0.0122$; t-test, Figure 2 f).

Figure 4 shows preening (grooming) daily profiles with a significantly higher duration (Figure 4 a, b) and frequency (Figure 4 d, e) during the day in both the light conditions. Two-way ANOVA revealed a significant effect of the time-of-day and condition × time-of-day interaction, but not of light condition, on hourly preening duration (light condition: $F_{1,230} = 4.778$, $p = 0.0718$; time-of-day: $F_{23,230} = 4.778$, $p < 0.0001$; condition × time-of-day interaction: $F_{23,230} = 2.595$, $p < 0.0002$; 2-way ANOVA) and frequency (light condition: $F_{1,230} = 4.017$, $p = 0.0729$; time-of-day: $F_{23,230} = 3.900$, $p < 0.0001$; condition × time-of-day interaction: $F_{23,230} = 1.749$, $p < 0.0212$; 2-way ANOVA). Both, the daily preening duration ($t_{10} = 2.013$, $p = 0.0718$; t-test, Figure 4 c) and frequency ($t_{10} = 2.004$, $p = 0.0729$; t-test, Figure 4 f) were not significantly different between the two light conditions.

Further, Rayleigh's test revealed that all the behavioural patterns were not uniformly distributed ($p < 0.05$) throughout the day in synchronized as well as the free running condition. Subsequently, all the behaviours were significantly concentrated during the day with r values ranging from 1 to 0.756 (Moore's modified Rayleigh's test). There was also a significant difference in the general activity ($U^2 = 0.216$, $p < 0.05$) and hopping ($U^2 = 2.82$, $p < 0.001$; Watson's U^2 test), but not in the ingestive or grooming behaviours, between the LD and LL_{dim} conditions.

Crows followed a diurnal pattern, with activity restricted during the day (LD) or subjective day (LL_{dim}), in all the behaviours monitored (Figures 1–4), conforming with characteristics of a diurnal species[10,11,15,16]. Correlated patterns of activity and ingestive behaviour (eating and drinking) suggest temporal proximity between the activity-driven functions, viz. movement/locomotion and foraging in crows. However, the activity was mainly restricted to the first half of the day, with low or absence of activity during the evening (cf. Figure 1–4) as indicated
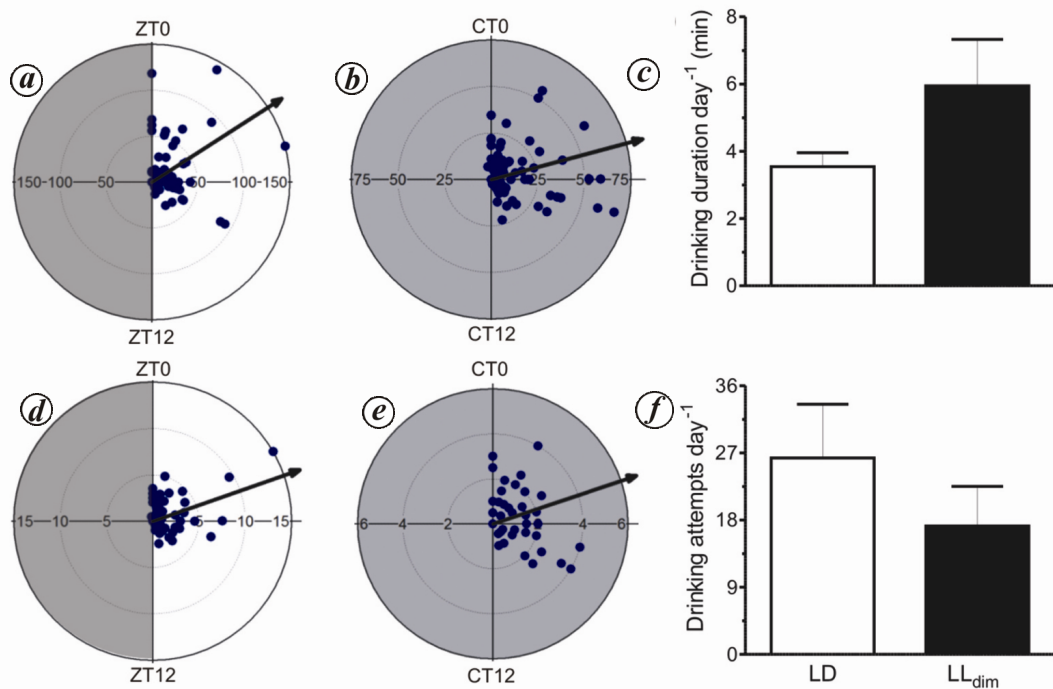
Activity behaviour

Figure 1. *a*, Representative actogram of an Indian house crow sequentially exposed to 12 h light : 12 h darkness (12 L : 12 D) and to constant dim light (LL_dim). Polar plots of general activity (*b*, *c*) and perch hop (*e*, *f*) distributions of each crow over 24 h under the 12 L : 12 D and LL_dim conditions. Arrow indicates the weighted mean time of the behaviour. *d* and *g*, Total general activity and perch hops respectively, plotted under 12 L : 12 D (white bar) and LL_dim (black bar) conditions. Asterisk on a bar indicates significant difference between the LD and LL_dim conditions (Students *t*-test, $p < 0.05$).

Figure 2. Eating behaviour of crows under the 12 L : 12 D and LL_dim conditions. Polar scatter plots of eating duration (*a*, *b*) and attempts (*d*, *e*) of each crow per hour over 24 h under the 12 L : 12 D (*a*, *d*) and LL_dim (*b*, *e*) conditions. Arrow indicates the weighted mean time of the behaviour. *c* and *f* are the total eating duration and attempts (mean ± SE) under the 12 L : 12 D (white bar) and LL_dim (black bar) conditions respectively. Asterisk on a bar indicates significant difference between the LD and LL_dim conditions (Students *t*-test, $p < 0.05$).

Figure 3. Drinking behaviour of crows under 12 L : 12 D and LL$_{dim}$ conditions. Polar scatter plots of drinking duration (*a*, *b*) and attempts (*d*, *e*) of each crow per hour over 24 h under the 12 L : 12 D (*a*, *d*) and LL$_{dim}$ (*b*, *e*). Arrow indicates the weighted mean time of the behaviour. *c* and *f* are the total drinking duration and attempts (mean ± SE) under the 12 L : 12 D (white bar) and LL$_{dim}$ (black bar) conditions respectively.
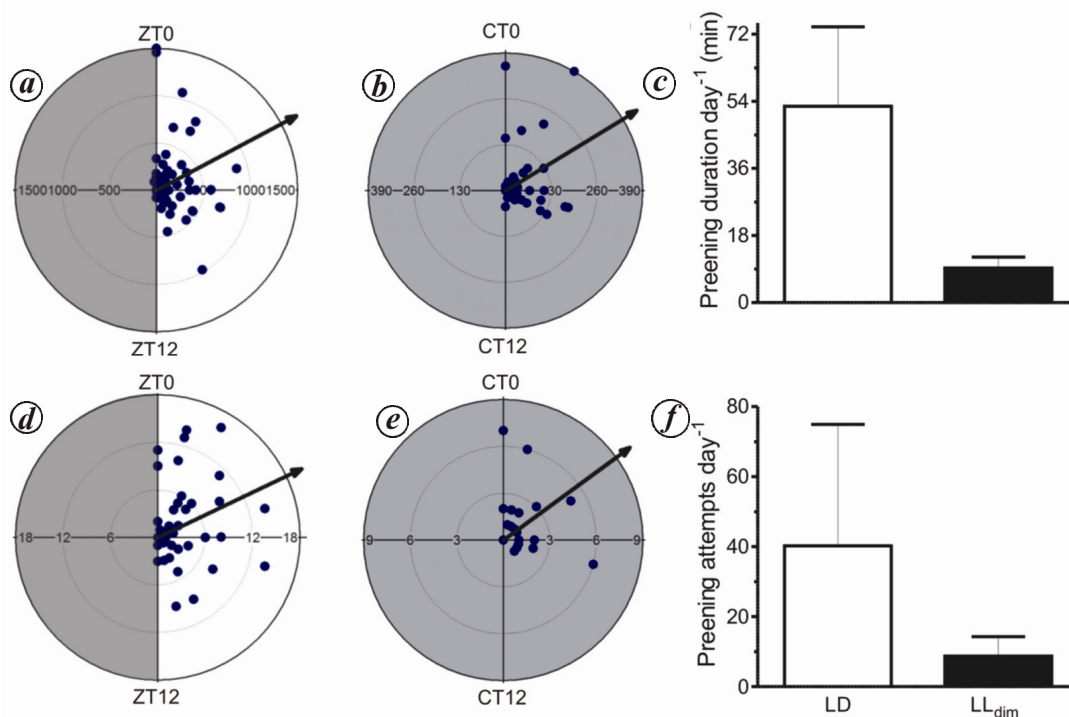
Figure 4. Preening behaviour of crows under 12 L : 12 D and LL$_{dim}$ conditions. Polar scatter plots of preening duration (*a*, *b*) attempts (*d*, *e*) of each crow per hour over 24 h under the 12 L : 12 D (*a*, *d*) and LL$_{dim}$ (*b*, *e*) conditions. Arrow indicates the weighted mean time of the behaviour. *c* and *f* are the total drinking duration and attempts (mean ± SE) under 12 L : 12 D (white bar) and LL$_{dim}$ (black bar) conditions respectively.

by the observations on foraging[13], predation[14] and sleep patterns[17] in corvids. However, this appears inconsistent with the general idea of two-oscillators (M and E; morning and evening) controlling the daily activity pattern[3,8], as reported in several species including birds[10,11,18]. A significantly higher locomotion and ingestive behaviour early in the day is nonetheless consistent with the idea that crows compensate for inactivity periods in preceding dark period in LD or subjective night in the LL_{dim} condition[19].

Interestingly, both the duration and frequency of preening were high early in the day (or subjective day) and attenuated by mid-day, the peak time of the ingestive behaviours (Figure 4). Also, unlike the other behavioural patterns (cf. Figure 1–4), preening was re-consolidated in the second half of the day, as has been reported in Zebra finches[16]. Thus, preening was not correlated with the activity and feeding behaviour patterns in crows, consistent with the reported negative relationship of preening to ingestion and locomotion in Zebra finches[16]. We reason that because preening is a defense mechanism and involved in the removal of ectoparasites, plumage maintenance, etc.[20], it is distinctly different from other behaviours in sharing a considerable proportion of the daytime. The time allocation for daily behaviours has been argued as a critical factor in maximizing the survival and fitness of birds, and appears to have evolved through the process of natural selection[7,11,21].

Persistence of behavioural patterns under LL_{dim} with a circadian period (Figure 1 a) suggests role of circadian clocks in determining daily behaviours in Indian house crows. We argue that a significantly attenuated activity levels under LL_{dim} were due to negative masking effects of the dim light intensity on the circadian outputs. At the same time, the absence of such suppresive effects on preening and feeding behaviours might suggest the involvement of separate regulatory pathways in regulating different functions. These activities are regulated either by separate circadian oscillators or by separate output pathways emanating from a common circadian pacemaker. It is also likely that compared to activity, circadian rhythms underlying feeding and preening behaviours are more robust, and hence not affected under the LL_{dim} condition. Further, feeding is critical for the energy homeostasis and so might be more tightly coupled to the endogenous circadian timing.

In conclusion, our results show association of activity with the ingestive behaviours, not grooming, in Indian house crows. We suggest a role of endogenous self-sustaining clock(s) in temporal organization of daily behaviours in crows, although with variable patterns as evidenced by differences in the robustness of circadian rhythms under the LL_{dim} condition.

1. Brandt, M. J. and Cresswell, W., Diurnal foraging routines in a tropical bird, the rock finch *Lagonosticta sanguinodorsalis*: how important is predation risk? *J. Avian Biol.*, 2009, **40**, 90–94.

2. Newton, I., *Population Limitation in Birds*, Academic Press, 1998.

3. Aschoff, J., Free running and entrained circadian rhythms. In *Handbook of Behavioral Neurobiology, Biological Rhythms* (ed. Aschoff, J.), Springer-Verlag, Berlin, New York, 1981, vol. 4, pp. 81–93.

4. Fuller, P. M., Gooley, J. J. and Saper, C. B., Neurobiology of the sleep–wake cycle: sleep architecture, circadian regulation, and regulatory feedback. *J. Biol. Rhythm*, 2006, **21**, 482–493.

5. Laposky, A. D., Bass, J., Kohsaka, A. and Turek, F. W., Sleep and circadian rhythms: key components in the regulation of energy metabolism. *FEBS Lett.*, 2008, **582**, 142–151.

6. Duffy, J. F. and Dijk, D. J., Getting through to circadian oscillators: why use constant routines? *J. Biol. Rhythm*, 2002, **17**, 4–13.

7. Helm, B. and Visser, M. E., Heritable circadian period length in a wild bird population. *Proc. R. Soc. London*, 2010, **B277**, 3335–3342.

8. Pittendrigh, C. S., Circadian systems: entrainment. In *Handbook of Behavioral Neurobiology, Biological Rhythms* (ed. Aschoff, J.), Springer-Verlag, Berlin, New York, 1981, vol. 4, pp. 239–268.

9. Benstaali, C., Mailloux, A., Bogdan, A., Auzeby, A. and Touitou, Y., Circadian rhythms of body temperature and motor activity in rodents: their relationships with the light-dark cycle. *Life Sci.*, 2001, **68**, 2645–2656.

10. Kumar, V., Singh, B. P. and Rani, S., The bird clock: A complex multi-oscillatory and highly diversified system. *Biol. Rhythm Res.*, 2004, **35**, 121–144.

11. Cassone, V. M., Avian circadian organization: a chorus of clocks. *Front. Neuroendocrinol.*, 2014, **35**, 76–88.

12. Clayton, N. S. and Dickinson, A., Scrub jays (*Aphelocoma coerulescens*) remember the relative time of caching as well as the location and content of their caches. *J. Comp. Psychol.*, 1999, **113**, 403.

13. Engel, K. A. and Young, L. S., Daily and seasonal activity patterns of common raven in southwestern Idaho. *Willson Bull.*, 1992, **104**, 462–471.

14. Bayne, E. M. and Hobson, K. A., Temporal pattern of predation on artificial nest in the southern boreal forest. *J. Wildlife Manage.*, 1997, **61**, 1127–1234.

15. Ruchi and Malik, S., Time allocation of daily behaviours in subtropical passerine finch, spotted munia (*Lonchura punctulata*). *Biol. Rhythm Res.*, 2015, **46**, 941–947.

16. Ollason, J. C. and Slater, P. J., Changes in the behaviour of the male zebra finch during a 12-hr day. *Anim. Behav.*, 1973, **21**, 191–196.

17. Szymczak, J. T., Influence of environmental temperature and photoperiod on temporal structure of sleep in corvids. *Acta Neurobiol. Exp.*, 1989, **49**, 359–366.

18. Daan, S. and Aschoff, J., Circadian rhythms of locomotor activity in captive birds and mammals: their variations with season and latitude. *Oecologia*, 1975, **18**, 269–316.

19. Beer, J. R., Winter feeding patterns in the house sparrow. *Auk*, 1961, **78**, 63–71.

20. Delius, J. D., A stochastic analysis of the maintenance behaviour of skylarks. *Behaviour*, 1969, **33**, 137–177.

21. Yerushalmi, S. and Green, R. M., Evidence for the adaptive significance of circadian rhythms. *Ecol. Lett.*, 2009, **12**, 970–981.