Entrainment of circadian rhythms of locomotor activity by ambient temperature cycles in the dromedary camel

Hicham Farsi1, Mohamed R. Achaâban2, Mohammed Piro2, Béatrice Bothorel3, Mohammed Ouassat1, Etienne Challet3, Paul Pévet3 & Khalid El Allali1*

In the dromedary camel, a well-adapted desert mammal, daily ambient temperature (Ta)-cycles have been shown to synchronize the central circadian clock. Such entrainment has been demonstrated by examining two circadian outputs, body temperature and melatonin rhythms. Locomotor activity (LA), another circadian output not yet investigated in the camel, may provide further information on such specific entrainment. To verify if daily LA is an endogenous rhythm and whether the desert Ta-cycle can entrain it, six dromedaries were first kept under total darkness and constant-Ta. Results showed that the LA rhythm free runs with a period of 24.8–24.9 h. After having verified that the light–dark cycle synchronizes LA, camels were subjected to a Ta-cycle with warmer temperatures during subjective days and cooler temperatures during subjective nights. Results showed that the free-running LA rhythm was entrained by the Ta-cycle with a period of exactly 24.0 h, while a 12 h Ta-cycle phase advance induced an inversion of the LA rhythm and advanced the acrophase by 9 h. Similarly, activity onset and offset were significantly advanced. All together, these results demonstrate that the Ta-cycle is a strong zeitgeber, able to entrain the camel LA rhythm, hence corroborating previous results concerning the Ta non-photic synchronization of the circadian master clock.

In order to survive, mammals have evolved several adaptation strategies to cope with ecological pressures of their biotope. In order to escape or enter a synchronous state with biotic and abiotic environmental factors, animals regulate their 24-h general activity patterns to be nocturnal, diurnal, crepuscular or even cathemeral1–4. Thus, predation avoidance, availability of food and partners for reproduction, temperature and many other factors strongly modulate these time-partitioning strategies2,5–9. Defining the diel time partitioning of activity in a species and its entrainment by environmental cues requires tracking animal movements and clear experimental demonstrations. This is further complicated when we consider species living in harsh environments like deserts. In such biotopes, components like heat exposure, dehydration and food scarcity determine the survival of these animals and modulate their general activity, rendering its exact pattern hard to establish. The dromedary camel is a diurnal animal10,11 which is well-adapted to the hostile desert environment. It’s physiological processes of adaptation culminate in water economy. Among these mechanisms, adaptive heterothermia12,13 is certainly one of the most interesting phenomena. It is an exceptional thermoregulatory feature displayed by camels exposed to heat stress and dehydration in order to minimize water expenditure. Under such thermal regulation, ambient temperature (Ta) modulates the body temperature (Tb), as is the case in ectotherms (reptiles and amphibians), inducing daily fluctuations of Tb over a range of 6–8 °C. The desert Ta cycle has been shown to modulate another feature of camel adaptive physiology. Indeed, it has been demonstrated that the daily variation of Tb in the dromedary camel, like the LD cycle, is able to synchronize the central circadian clock by shifting two of its outputs, the rhythms of Tb and melatonin (Mel)14, the first clear demonstration of such temperature dependence in a mammal. This suggests that other circadian rhythms such as locomotor activity (LA) could also be entrained by desert Ta cycles. In the present study LA rhythms in dromedary camels under specific indoor experimental conditions

1Comparative Anatomy Unit, Department of Biological and Pharmacological Veterinary Sciences, Hassan II Agronomy and Veterinary Medicine Institute, Rabat-Instituts, BP: 6202, 10101 Rabat, Morocco. 2Medicine and Surgical Unit of Domestic Animals, Department of Medicine, Surgery and Reproduction, Hassan II Agronomy and Veterinary Medicine Institute, Rabat, Morocco. 3Institute of Cellular and Integrative Neurosciences, CNRS and University of Strasbourg, Strasbourg, France. *Email: k.elallali@iav.ac.ma
have been examined to verify if this rhythm is driven by the circadian clock and whether it is synchronized by light–dark (LD) and Ta cycles.

**Results**

**Dromedary camels exhibit a circadian rhythm of locomotor activity.** Under a cyclical environment of LD and an uncontrolled Ta cycle (stages 1 of both experiments 1 and 2), camels’ LA shows a clear daily rhythm with a period of exactly 24.0 h ($P \leq 0.05$) and a high robustness level of 37 to 42% (Figs. 1, 2, Table 1). The activity started early in the photophase and then reached its peak in the middle of the day when acrophase has been shown to occur, around 12:50 h. Thereafter, activity dropped and almost disappeared during the scotophase. Actograms confirm that the high levels of activity, corresponding to scores of 2, 3 and 4, coincide within the light phase/high Ta, while the lack of activity (scores of 0 and 1) is limited to the dark phase/low Ta.

During stages 2 (experiment 1 and 2), the LA rhythm persisted in the absence of any temporal cue. Indeed, compared to the daily LA rhythm (24.0 h period) first shown under cyclic indoor conditions of stage 1, camels...
transferred to constant conditions of stages 2 displayed a circadian rhythm of LA with a period completely different from 24.0 h, respectively of 24.8 ± 0.07 h and 24.9 ± 0.03 h for experiments 1 and 2. The one-way ANOVA confirms the existence of very significant differences (P ≤ 0.001) between the circadian period and the period calculated for stages 1. Moreover, a classical free-running diminution in rhythm regularity was noticed in stages 2. This was underlined by the drop (P ≤ 0.05) in robustness values to a range of 16 to 17% (Table 1). Likewise, the mean acrophase of all camels calculated for the last day of stages 2 showed a shift by almost 10 to 16 h to take place at 23:18 ± 0.70 h and 04:39 ± 1.84 h, respectively, for experiments 1 and 2. Visual inspection of the actograms (Figs. 1, 2) shows a daily drift (phase delay) of LA, confirming its free-running state. As highlighted in Table 1, both mesor and amplitude also underwent significant changes. These results, together with the persistence of rhythmicity under constant conditions, indicate clearly the existence of a circadian clock in the dromedary camel that drives the LA rhythm.

**Light–dark cycle entrainment.** When camels were subjected to a cyclic environment with 12L:12D cycle (stage 3 of experiment 1), LA rhythm became more regular and perfectly synchronized with the LD cycle (Fig. 1). This was highlighted by an increase of robustness which reached 39.7 ± 2.1% (Table 1). Likewise, the circadian period (24.8 h) of the former free-running conditions (Stage 2) was shortened to become exactly 24.0 h, equal to the LD cycle period. Significant differences (P ≤ 0.05) occurred regarding these changes of the period through stages 1, 2 and 3.

Actograms (Fig. 2) show that the LD cycle induced a daily progressive phase advance in activity. It required 2 to 3 days to reach a perfect resynchronization with the new light regime. Meanwhile, the acrophase was significantly (P ≤ 0.05) advanced by almost 5 h to occur at 12:50 ± 0.09 h instead of 16:45 ± 0.53 h, recorded in the previous stage. The applied LD cycles also induced an increase in the mesor and amplitude (Table 1). All these results clearly indicate that the exposure of camel to an LD cycle following DD conditions results in the synchronization of LA rhythms.

A phase delay of 6-h in the light regime during stage 4 lead to a phase delay in the LA rhythm, but took a few days to occur (Fig. 1). This shift in activity is corroborated by the Cosinor regression showing a significant (P ≤ 0.05) delay in acrophase by almost 6 h, the acrophase occurred at 17:44 ± 0.19 h instead of 12:50 ± 0.09, as observed in the previous stage. The other parameters remained unchanged (Table 1). These findings illustrate that LA rhythm in the dromedary camel is circadian and entrained by the light–dark cycle.

**Ambient temperature cycle entrainment.** The circadian nature of LA rhythm and its entrainment by LD cycle having been established, the next step was then to verify whether Tₐ cycle is a zeitgeber capable of entraining this rhythm in the camel. When animals were placed under a 24-h Tₐ cycle environment (10-h of warmer temperature and 9-h of cooler temperature) (NRT, Stage 3, experiment 2) with heating during the subjective day and cooling during the subjective night, a robust rhythm of activity was recorded (Fig. 2, Supplementary Fig. S1) with a period of exactly 24.0 h. This period was significantly (P ≤ 0.05) different from that of the previous stage (constant conditions, τ = 24.9 ± 0.03 h). Furthermore, an improvement in rhythm regularity was noticed (Fig. 2), with an increased robustness reaching 29.6 ± 0.75% (Table 1). These changes suggest that the applied Ta cycle imposes its own period and shape to the LA rhythm. Indeed, actograms showed that high activity levels were synchronized to the warmer period of Tₐ cycle, while inactivity coincides with the cooler period (Fig. 2). The acrophases occurred at 12:19 ± 0.31 h (Table 1).

The reversal of the Tₐ cycle (RT, 12-h phase advance) in stage 4 (experiment 2) resulted in a significant (P ≤ 0.05) total inversion (12-h phase advance) of the LA rhythm (Fig. 2, Supplementary Fig. S1). This phase shifting occurred within a few days, requiring 9.03 ± 1.01 days to be fully synchronized with the new Tₐ regime. The peak of activity switched to occur during the subjective night, which corresponded to the warmer period of the new applied Tₐ cycle. ANOVA analysis showed a significant (P ≤ 0.05) advance in the acrophase of LA, which occurred at 03:19 ± 0.23 h (Table 1). As for the acrophase, the activity onset and offset of both stages 3 and 4 confirmed the existence of a phase relationship with the Tₐ cycles. A significant phase advance (P ≤ 0.05) of both onsets and offsets were seen when Tₐ was changed from the NRT cycle (stage 3) to a RT cycle (stage 4).

### Table 1. Comparison of the LA rhythm parameters (mean ± SEM) between different stages of experiments 1 and 2. *Acrophases under constant conditions were calculated for the last day of stages 2.

| Experiment 1 | Period      | Acrophase     | Mesor       | Amplitude | Robustness (%) |
|--------------|-------------|---------------|-------------|-----------|----------------|
| Stage 1      | 24:0 ± 0.02 h | 12:52 ± 0.14 h | 1:70 ± 0.05 | 1:43 ± 0.06 | 42.9 ± 1.50    |
| Stage 2      | 24:8 ± 0.07 h | 23:18 ± 0.70 h | 0:09 ± 0.09 | 0:75 ± 0.03 | 17:3 ± 1.14    |
| Stage 3      | 24:0 ± 0.02 h | 12:50 ± 0.09 h | 1:71 ± 0.04 | 1:41 ± 0.01 | 39:7 ± 2.10    |
| Stage 4      | 24:0 ± 0.05 h | 17:44 ± 0.19 h | 1:67 ± 0.05 | 1:47 ± 0.06 | 40:1 ± 1.50    |
| Experiment 2 |             |               |             |           |                |
| Stage 1      | 24:0 ± 0.02 h | 12:55 ± 0.10 h | 1:8 ± 0.09  | 1:50 ± 0.06 | 37.2 ± 1.30    |
| Stage 2      | 24:9 ± 0.03 h | 04:39 ± 1.84 h | 0:94 ± 0.04 | 0:94 ± 0.06 | 16:6 ± 0.61    |
| Stage 3      | 24:0 ± 0.03 h | 12:19 ± 0.31 h | 1:53 ± 0.09 | 1:01 ± 0.04 | 29:6 ± 0.75    |
| Stage 4      | 24:0 ± 0.04 h | 03:19 ± 0.23 h | 1:41 ± 0.04 | 1:00 ± 0.03 | 25:0 ± 0.90    |
that the Ta cycle is a strong zeitgeber, able to entrain the LA rhythm in the dromedary camel. In fact, it seems that entrainment by the Ta cycle of the molecular machinery of the central clock is now well established. By contrast, much less is known about the neuronal process of non-photic entrainment of the circadian clock. Our results in experiment 2 showed that, in the absence of photic entrainment (DD conditions), a 24.0 h artificial Ta cycle with a warmer period during the subjective day and cooler period during the subjective night was able to entrain the free running rhythm of LA of camels. A 12-h phase advance in the daily Ta cycle with the warmer period changed to the subjective night and cooler period to the subjective day induced a complete shift (almost 12 h phase advance) in LA rhythm. Whatever the applied Ta cycle, the maximum of camel activity always coincided with the warmer period. This corroborates previous observations made in this species under natural conditions which characterized its diurnality, acrophase of LA occurring during daytime.

All these results confirm that, in the absence of a photic signal, Ta cycle is a strong zeitgeber capable of entraining the LA rhythm in the dromedary camel. However, one can argue that such entrainment of LA rhythms is not specific to camels. Across the literature, several studies have emphasized the effect of the Ta cycle on general rest-activity rhythm in various animals, especially non-mammalian vertebrates. Whereas in mammals, only partial entrainment of LA rhythm has been reported in some species, including squirrel monkeys, marmosets, palm squirrels, and mice. Regarding these results, it is quite difficult to distinguish a real entrainment of the central circadian clock from a masking effect on the LA rhythm. In the camel, we have previously shown that the circadian clock is synchronized by Ta cycles since two robust outputs of the clock, Tb and Mel rhythms, are entrained by Ta cycles. The observed entrainment of LA rhythm in the present study corroborates these results and confirms the entrainment of the camel SCN by this non-photic cue.

Under heat stress and dehydration, the camel displays an adaptive heterothermy consisting of a switch from a perfect endothermy state to an ectothermy state. Indeed, a fully hydrated camel is a perfect endothermic-homeotherm species with constant body temperature not exceeding a daily variation of 2 °C. However, when dehydrated and subjected to excessive heat load of the desert, the camel becomes heterothermic, functioning like ectothermic-poikilotherms (reptiles, amphibians) with Tb passively following the Ta cycle. Thus, during the day camels store the heat, but during the night when the thermal gradient becomes negative (Tb lower than Ta), heat is dissipated passively by convection and conduction. Heterothermic camels display daily variations in Tb that are life threatening for other mammals, with morning records about 34 °C and evening values of 42 °C. This phenomenon is one of the most fascinating adaptive processes to cope with the extreme conditions of the desert. Indeed, adaptive heterothermy allows water economy by preventing the use of evaporative cooling mechanisms that are water consuming. It was reported that this specific thermoregulation state allows a dromedary of 600 kg of body weight to save up to 6 L of water/day.

The particular Ta synchronization of the circadian system in the camel may be related to its specific thermoregulatory system and adaptation to the desert habitat. Possible circadian entrainment by Ta depends on the type of thermoregulation displayed by a species. This classifies animals into two categories: ectothermic-poikilotherms in which temperature cycle is a strong zeitgeber capable of synchronizing the central circadian clock, and endothermics-homeotherms for whom Tb has a weak effect on the circadian system (For review see Ref). In this general rule, a third type of species is added, the heterotherm species for whom Tb has similar effect on the circadian system as in ectothermic-poikilotherm. In fact, it seems that entrainment by Ta cycle require a specific sensitivity to Tb changes, present in ectothermic-poikilothermic and heterothermic species, such as dromedary camels. Recently, heterothermy has also been reported to occur, to some extent, in other desert ungulates like oryx and goat. This could explain why the circadian system of the desert black Moroccan goat, displays similar entrainment of Tb as is seen in the camel. Accordingly, three outputs of the goat's central circadian clock, namely the Tb, LA and Mel rhythms are entrained by Tb cycle.

Adaptation to the desert for large mammals like camels, that cannot burrow to avoid heat stress as can small animals, would require coping with the Ta cycle by using several strategies. In addition to heterothermy that allows specific thermoregulation and economizing of water, this species could use Ta to modulate its circadian physiology. Camels seem to be able to shift the timing of their daily activities depending on the Ta cycle. This mechanism is employed by some desert ungulates to reduce heat loads and minimize water loss during the hottest seasons. It was reported that during the winter season, dromedaries graze during the day and rest at night; while during hot seasons and under solar radiation stress, camels seek shade and become inactive. Such temporal niche switching of activity was demonstrated and intensively investigated in the Arabian oryx, desert bighorn sheep, desert mule deer and other desert mammals. During the hot season, the circadian rhythm of activity in the Arabian oryx switches from diurnal to crepuscular or even nocturnal. This specific day-time inactivity was demonstrated to be a NREM sleep.
Figure 3. Shift in onset and offset of the LA rhythm (dots with error bars: Means ± SEM) by a phase advance of $T_c$ cycle in DD conditions of experiment 2 (camels, $n=6$). (a,b) represent, respectively, the means of onsets and offsets during stage 3 (DD + NRT) in which camels were first maintained under an artificial daily $T_c$ cycle with a peak of heat during the subjective day. (c,d) represent, respectively, the means of onsets and offsets during stage 4 (DD + RT) in which camels were placed under a 12-h advanced $T_c$ cycle with a peak of heat during the subjective night. Blue (with blue thermometer symbol) and red (with red thermometer symbol) areas indicate the cold and heat periods of each $T_c$ cycle.
Daily changes in environmental cues, specifically the LD cycle, are commonly used as a predictive and stable external factor for the precise measurement of time throughout the year. Hence, it is a reliable cue for mammals to drive and modulate seasonal rhythms such as reproductive behavior, migration, moulting…which thus occur in the optimal season. However, in desert regions changes in photoperiod are less important than at high latitudes. Thus, in some regions an annual variation of only 1-h is observed whilst in high latitudes it can reach 14-h. Although desert animals like camels seem able to integrate even low variations in photoperiod23, this alone would not be strong enough to drive seasonal functions. In this regard a strong environmental cue such as T_s could be important for driving such rhythms. As previously reported, T_s in the desert is known to be a dominant environmental cue able to affect different physiological processes and behavior. At a seasonal rhythmicity level, there are two or three examples in which T_s has been experimentally demonstrated to drive seasonal rhythms. In *Spermophilus tridecemlineatus* and *Spermophilus lateralis* two squirrel species living in both forested and arid areas of North America, T_s was shown to be a strong zeitgeber much more powerful than the photoperiodic, able to modulate and shift circannual rhythms like body weight, hibernation and reproductive activity63–66. T_s seems also to be important for maintaining seasonal rhythms in two examples of small animals, the European hamster and the edible dormouse (*Glis glis*), in which under constant photoperiodic regimes (LD or LL), the circannual rhythms of testosterone, thyroxine and activity are present under cyclic T_s while they disappear when T_s was constant67–69. Such demonstrations are unique among mammals because to the best of our knowledge there have been no other attempts to highlight similar findings in other species. Such protocols are difficult to conduct, especially on large mammals such as camels and goats since they are time consuming (2–3 years/breeding cycles), costly and technically difficult, as controlling T_s stability for years is complicated.

The desert is a distinctive habitat, in which camels and other ungulates have to face the heat and the T_s effects by employing reparatory adaptive mechanisms (heterothermy, renal reabsorption…) but also by using their sensitivity to T_s to permit anticipatory adaption. With LD cycles, T_s in the desert shapes the activity of animals and defines the temporal division of this circadian clock for maintaining energy balance and water economy, specifically during the driest and hottest time of the day. This is probably one of the reasons for which species like the camel have to have a circadian system that can be entrained by T_s and shows flexibility to tolerate and avoid the unpredictable environmental conditions that can result in heat stress and dehydration.

**Conclusion**

To date the T_s cycle entrainment ability of the circadian master clock has been described in only two mammalian species, the camel14 and recently in the desert goat15. The results of the present work show clearly that locomotor activity, another output of the master circadian clock, is also entrained in the camel by the daily T_s cycles. This corroborates the fact that T_s daily cycle is a strong environmental cue in the desert habitat, capable of synchronizing the central circadian clock of the camel and the goat. Together, these findings suggest that other desert mammalian species would likely be endowed with a specific circadian system which is modulated by the desert T_s cycle variations. Further investigations are still needed to elucidate this hypothesis.

**Methods**

**Animals.** Six non-pregnant adult female camels (6–9 year) originating from southern Morocco (latitude 23° 43’ N, 15° 57’ S) were used for this study. Animals were housed in specific facilities at the Hassan II Agronomy and Veterinary Medicine Institute of Rabat (Latitude: 34° 01’ N, Longitude: 6° 50’ W). They received a compound feed (Maraa for Camelids, Alf Sahel, Morocco) and barley straw ad libitum and had free access to water. All animal procedures adopted in the present study comply with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. The study was in agreement with the Hassan II Agronomy and Veterinary Institute of Rabat and Moroccan Ministry of Agriculture recommendations which are in accordance with international ethical standards (European Union Directive 2010/63/EU) legislation and recommendations in the field of chronobiology68.

**Experimental design.** *Experiment 1* was designed to determine whether the LA rhythm in camel is driven by a circadian clock and also to demonstrate the entrainment of this rhythm by the LD cycle. The light intensity was 500 lx. Camels were housed individually and were able to move freely in a controlled sheltered stable of 40 m². This experiment was carried out on animals one by one. Each individual experiment consisted of four stages totalling 73 successive days,

- **Stage 1** (7 days) was an adaptive period of camels to the new indoor conditions. An artificial LD cycle of 12L:12D was applied with an uncontrolled T_s (18–24 °C) cycle.
- **Stage 2** (26 days) This step was intended to demonstrate that the LA rhythm is endogenous. Camels were maintained under constant conditions of total darkness (DD) and constant ambient temperature (CT) of 23.0 ± 0.58 °C to prevent any environmental cyclicity that could provide temporal cues.
- **Stage 3** (22 days) The CT conditions (23.0 ± 0.7 °C) were maintained and an LD cycle of 12L:12D was applied with lights switched on at 07:00 AM.
- **Stage 4** (18 days) The same conditions as stage 3 were maintained; however, a phase delay of + 6 h was applied to the LD cycle; lights were switched on at 01:00 PM.

*Experiment 2* was conducted to verify if T_s cycles are able to entrain LA rhythms in the camel. Similar to the previous experiment (experiment 1), camels were housed individually and allowed to move freely in the same
controlled sheltered stable of 40 m². This experiment was also conducted on each individual camel (one by one). One individual experiment lasted 61 successive days and consisted of the following stages:

- **Stage 1 (5 days)** was designed to adapt camels to indoor conditions before starting the following stages. An artificial LD cycle of 12L:12D (light intensity of 500 lx) and a CTₐ of 18.5–25 °C were applied.
- **Stage 2 (20 days)** As in stage 2 of experiment 1, camels were placed under constant conditions with DD and a CTₐ of 23.0 ± 0.5 °C.
- **Stage 3 (15 days)** DD conditions were maintained and a Tᵥ cycle (NRT: non reversed temperature) was applied with a warmer period (32.5 °C for about 10 h) during the subjective day and the cooler period (16 °C for almost 9 h) during the subjective night. The ascending and descending phases of the Tᵥ cycle in which temperatures gradually and respectively increased and decreased were about 2h30min each.
- **Stage 4 (21 days)** DD conditions were kept while the Tᵥ cycle was reversed (RT), with a 12-h phase advance to obtain the warmer period (31.9 °C for about 10 h) of the cycle during the subjective day and the cooler period (16.2 °C for almost 10 h) during the subjective day. The ascending and descending phases of Tᵥ cycle were about 2 h each.

**Locomotor activity recording.** The rhythm of LA was recorded individually in camels using a validated video-locomotion scoring technique15. The recorded video sequences were visually analyzed by two well-trained evaluators who assigned 6 scores to each activity state following a predefined scale where:

- Score 0 correspond to a camel in a sitting position and not moving.
- Score 1 is a sitting position with slight movements.
- Score 2 represents a camel in standing position but not moving.
- Score 3 is a standing camel just moving its limbs with no real locomotion.
- Score 4 corresponds to a camel walking with exploration of the surrounding area; and
- Score 5 is assigned to a camel with intense locomotor activity or in an agitated state.

As previously reported15, a time set of 5 min was chosen to record LA rhythm in dromedary camels.

**Data analysis.** The daily profiles of LA were plotted using Sigma-Plot software (SigmaPlot v12.0, Systat, Chicago, IL). The actograms were plotted using the software Actogram Plotter (Refinetti R, Circadian Rhythm Laboratory, University of South Carolina, https://www.circaadian.org/software.html).

The LA rhythm parameters, including period, acrophase, mesor, amplitude and robustness, were calculated using nonlinear least squares method with the help of the following software: Cosinor, Acro and LSP software (Refinetti R, Circadian Rhythm Laboratory, University of South Carolina, https://www.circaadian.org/software.html). This Cosinor analysis was carried out according to the equation:

\[ f = y_0 + a \cdot \cos \left( 2\pi \left( t - \phi \right)/\tau \right) \]

where \( f \) is LA at time \( t \), \( y_0 \) is the mesor, \( a \) is the amplitude, \( \phi \) is the acrophase and \( \tau \) is the period. For each parameter, a 95% probability confidence interval is given. Likewise, the degree of significance of the regression was calculated.

Activity onsets and offsets were also determined. The onset represents the phase angle difference in minutes between the time of the beginning of the activity and the time of the cold–hot transition, i.e., time point at which 50% of the ascending phase of Tᵥ cycle was reached. While the offset is the phase-angle difference in minutes between the time of the end of activity and the time of the hot–cold transition, i.e., time at half of the Tᵥ descending phase.

A one-way ANOVA analysis followed by the Holm-Sidak post-hoc test was used for the statistical comparison of LA rhythm parameters between the different stages of experiments 1 and 2 and also to test the equality of means of the daily activity onsets and offsets around the daily Tᵥ transitions of NRT (stage 3) and RT (stage 4) cycles of experiment 2. \( P \leq 0.05 \) was considered statistically significant in all statistical tests.

Received: 12 June 2020; Accepted: 29 October 2020
Published online: 11 November 2020

**References**
1. Aschoff, J. Circadian activity pattern with two peaks. *Ecology* 47, 657–662 (1966).
2. Daan, S. & Aschoff, J. Circadian rhythms of locomotor activity in captive birds and mammals: Their variations with season and latitude. *Oecologia* 18, 269–316 (1975).
3. Katandukula, J. V., Bennett, N. C., Chimimba, C. T., Paulkes, C. G. & Oosthuizen, M. K. Locomotor activity patterns of captive East African root rats, *Tachyoryctes splendens* (Rodentia: Spalacidae), from Tanzania, East Africa. *J. Mamm.* 94, 1393–1400 (2013).
4. Bennie, J. J., Duffy, J. P., Inger, R. & Gaston, K. J. Biogeography of time partitioning in mammals. *Proc. Natl. Acad. Sci. U.S.A.* 111, 13727–13732 (2014).
5. Cloudsley-Thompson, J. L. *Rhythmic Activity in Animal Physiology and Behaviour* (Academic Press, Cambridge, 1961).
6. Aschoff, J., Gercke, H., Pohl, P., Rieger, P. V. & Wever, S. P. U. R. Interdependent parameters of circadian activity rhythms in birds and man. In *Biochronometry* (ed. Menaker, M.) 3–29 (National Academy of Science, Washington, DC, 1971).
7. Risenhoover, K. L. Winter activity patterns of moose in interior Alaska. *J. Wildl. Manage.* 50, 727–734 (1986).
8. Castillo-Ruiz, A., Paul, M. J. & Schwartz, W. J. In search of a temporal niche: Social interactions. *Prog. Brain Res.* 199, 267–280 (2012).
9. Kronfeld-Schorch, N., Visser, M. E., Salis, L. & van Gillis, J. A. Chronobiology of interspecific interactions in a changing world. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. https://doi.org/10.1098/rstb.2016.0248 (2017).
10. Farsi, H. et al. Validation of locomotion scoring as a new and inexpensive technique to record circadian locomotor activity in large mammals. Helyxion 4, e00980–e00980 (2018).
11. El Allali, K. et al. Smartphone and a freely available application as a new tool to record locomotor activity rhythm in large mammals and humans. Chronobiol. Int. 36, 1047–1057 (2019).
12. Schmidt-Nielsen, K., Schmidt-Nielsen, B., Jarnum, S. A. & Houpt, T. R. Body temperature of the camel and its relation to water economy. Am. J. Physiol. 188, 103–112 (1957).
13. Bouâouda, H. et al. Daily regulation of body temperature rhythm in the camel (Camelus dromedarius) exposed to experimental desert conditions. Physiol. Rep. 2, e12151 (2014).
14. El Allali, K. et al. Entrainment of the circadian clock by daily ambient temperature cycles in the camel (Camelus dromedarius). Am. J. Physiol. Regul. Integr. Comp. Physiol. 304, R1044–R1052 (2013).
15. Farsi, H. et al. Melatonin rhythm and outputs of the master circadian clock in the desert goat (Capra hircus) are entrained by daily cycles of ambient temperature. J. Pineal Res. 68, e12634 (2020).
16. Ebling, F. J., Lincoln, G. A., Wollnik, F. & Anderson, N. Effects of constant darkness and constant light on circadian organization and reproductive responses in the ram. J. Biol. Rhythms 3, 365–384 (1988).
17. Johnson, R. F., Randall, S. & Randall, W. Freerunning and entrained circadian rhythms in activity, eating and drinking in the cat. J. Interdiscipl. Cycle Res. 14, 315–327 (1983).
18. Ilge, B., Hörmicke, H. & Stähle, H. Circadian rhythms of rabbits during restricted feeding. Am. J. Physiol. 253, R46–R54 (1987).
19. Decourcy, G. & Decourcy, P. J. Adaptive aspects of activity rhythms in bats. Biol. Bull. 126, 14–27 (1964).
20. Erkert, H. G., Nagel, B. & Stephan, I. Light and social effects on the free-running circadian activity rhythm in common marmosets (Callithrix jacchus; Primates): Social masking, pseudo-splitting, and relative coordination. Behav. Ecol. Sociobiol. 18, 443–452 (1986).
21. O’Reilly, H., Armstrong, S. M. & Coleman, G. J. Restricted feeding and circadian activity rhythms of a predatory marsupial, Dasyurides byrnei. Physiol. Behav. 38, 471–476 (1986).
22. Boulou, Z., Frim, D. M., Dewey, L. K. & Moore-Ede, M. C. Effects of restricted feeding schedules on circadian organization in squirrel monkeys. Physiol. Behav. 45, 507–515 (1989).
23. Mahoney, M., Bult, A. & Smale, L. Phase response curve and light-induced fos expression in the suprachiasmatic nucleus and adjacent hypothalamus of Arvicanthis niloticus. J. Biol. Rhythms 16, 149–162 (2001).
24. Alagali, A. N., Bennett, N. C., Amor, N. M. & Hart, D. W. The locomotory activity patterns of the arid-dwelling desert hedgehog, Paracynus aethiopicus, from Saudi Arabia. J. Arid Environ. 177, 104141 (2020).
25. Verwey, M., Robinson, B. & Amir, S. Recording and analysis of circadian rhythms in running-wheel activity in rodents. J. Vis. Exp. https://doi.org/10.3791/50186 (2013).
26. Refinetti, R. Early research on circadian rhythms. In Circadian Physiology 2nd edn (ed. Refinetti, R.) 1–667 (CRC Taylor and Francis Group, Boca Raton, 2006).
27. Goldman, B. D., Goldman, S. L., Riccio, A. P. & Terkel, J. Circadian patterns of locomotor activity and body temperature in blind mole-rats (Spalax ehrenbergi). J. Biol. Rhythms 12, 348–361 (1997).
28. Kopp, C. et al. Effects of a daylight cycle reversal on locomotor activity in several inbred strains of mice. Physiol. Behav. 63, 577–585 (1998).
29. Giannetto, C., Casella, S., Caola, G. & Piccione, G. Photic and non-photic entrainment on daily rhythm of locomotor activity in goats. Anim. Sci. J. 81, 121–128 (2010).
30. Piccione, G., Giannetto, C., Casella, S. & Caola, G. Daily locomotor activity in five domestic animals. Anim. Biol. 60, 15–24 (2010).
31. Challet, E. Minireview: Entrainment of the suprachiasmatic clockwork in diurnal and nocturnal mammals. Endocrinology 148, 5648–5655 (2007).
32. Dibner, C., Schibler, U. & Albrecht, U. The mammalian circadian timing system: Organization and coordination of central and peripheral clocks. Annu. Rev. Physiol. 72, 517–549 (2010).
33. Tanaka, M., Ichitani, Y., Okamura, H., Tanaka, Y. & Ibata, Y. The direct retinal projection to VIP neuronal elements in the rat SCN. Brain Res. Bull. 31, 637–640 (1993).
34. Jacomy, H., Burlet, A. & Bosler, O. Vasoactive intestinal peptide neurons as synaptic targets for vasopressin neurons in the suprachiasmatic nucleus. Double-label immunocytochemical demonstration in the rat. Neuroscience 88, 859–870 (1999).
35. Aton, S. J., Cobwell, C. S., Harmar, A. J., Waschek, J. & Herzog, E. D. Vasoactive intestinal polypeptide mediates circadian rhythmicity and synchrony in mammalian clock neurons. Nat. Neurosci. 8, 476–483 (2005).
36. Reppert, S. M. & Weaver, D. R. Comparing clockworks: Mouse versus Drosophila. J. Biol. Rhythms 15, 357–364 (2000).
37. Reppert, S. M. & Weaver, D. R. Molecular analysis of mammalian circadian rhythms. Annu. Rev. Physiol. 63, 647–676 (2001).
38. Shearman, L. P. et al. Interacting molecular loops in the mammalian circadian clock. Science 288, 1013–1019 (2000).
39. Okamura, H., Yamaguchi, S. & Yagita, K. Molecular machinery of the circadian clock in mammals. Cell Tissue Res. 309, 47–56 (2002).
40. Takahashi, J. S., Hong, H. K., Ko, C. H. & McDearmon, E. L. The genetics of mammalian circadian order and disorder: Implications for physiology and disease. Nat. Rev. Genet. 9, 764–775 (2008).
41. Mohawk, J. A., Green, C. B. & Takahashi, J. S. Central and peripheral circadian clocks in mammals. Annu. Rev. Neurosci. 35, 445–463 (2012).
42. Rensing, L. & Rusoff, P. Temperature effect on entrainment, phase shifting, and amplitude of circadian clocks and its molecular bases. Chronobiol. Int. 19, 807–864 (2002).
43. Aschoff, J. & Tokura, H. Circadian activity rhythms in squirrel monkeys: Entrainment by temperature cycles 1. J. Biol. Rhythms 1, 91–99 (1986).
44. Pálková, M., Sigmund, L. & Erkert, H. G. Effect of ambient temperature on the circadian activity rhythm in common marmosets, Callithrix jacchus (primates). Chronobiol. Int. 16, 149–161 (1999).
45. Rajaratnam, S. M. W. & Redman, J. R. Entrainment of activity rhythms to temperature cycles in diurnal palm squirrels. Physiol. Behav. 63, 271–277 (1998).
46. Refinetti, R. Entrainment of circadian rhythm by ambient temperature cycles in mice. J. Biol. Rhythms 25, 247–256 (2010).
47. van Jaarsveld, B., Bennett, N. C., Hart, D. W. & Oosthuizen, M. K. Locomotor activity and body temperature rhythms in the Mahali mole-rat (C. h. mahali): The effect of light and ambient temperature variations. J. Therm. Biol. 79, 24–32 (2019).
48. Schmidt-Nielsen, K. The physiology of the camel. Sci. Am. 201, 140–151 (1959).
49. Wu, H. et al. Camelid genomes reveal evolution and adaptation to desert environments. Nat. Commun. 5, 5188 (2014).
50. Samara, E. M. Unraveling the relationship between the topographic distribution patterns of skin temperature and perspiration response in dromedary camels. J. Therm. Biol. 84, 311–315 (2019).
54. Pohl, H. Temperature cycles as zeitgeber for the circadian clock of two burrowing rodents, the normothermic antelope ground squirrel and the heterothermic Syrian Hamster. *Biol. Rhythm Res.* 29, 311–325 (1998).
55. Cain, J. W., Krausman, P. R., Rosenstock, S. S. & Turner, J. C. Mechanisms of thermoregulation and water balance in Desert Ungulates. *Wildl. Soc. Bull.* 1973–2006(34), 570–581 (2006).
56. Mengistu, U., Dahlborn, K. & Olsson, K. Mechanisms of water economy in lactating Ethiopian Somali goats during repeated cycles of intermittent watering. *Anim. Int. J. Anim. Biosci.* 1, 1099–1107 (2007).
57. Gauthier-Pillers, H. Aspects of dromedary ecology and ethology. In *The Camelid* (ed. Cockrill, W. R.) (Scandinavian Institute of African Studies, Uppsala, 1984).
58. Miller, G. D., Cochran, M. H. & Smith, E. L. Nighttime activity of desert bighorn sheep. *Desert Bighorn Council Trans.* 28, 23–25 (1984).
59. Hayes, C. L. & Krausman, P. R. Nocturnal activity of female desert mule deer. *J. Wildl. Manage.* 57, 897–904 (1993).
60. Davimes, J. G. et al. Temporal niche switching in Arabian oryx (Oryx leucoryx): Seasonal plasticity of 24h activity patterns in a large desert mammal. *Physiol. Behav.* 177, 148–154 (2017).
61. Davimes, J. G. et al. Seasonal variations in sleep of free-ranging Arabian oryx (Oryx leucoryx) under natural hyperarid conditions. *Sleep* https://doi.org/10.1093/sleep/zsy038 (2018).
62. El Allali, K. et al. Seasonal variations in the nycthemeral rhythm of plasma melatonin in the camel (Camelus dromedarius). *J. Pineal Res.* 39, 121–128 (2005).
63. Mrosovsky, N. Circannual cycles in golden-mantled ground squirrels: Phase shift produced by low temperature. *J. Comp. Physiol.* 136, 349–353 (1980).
64. Mrosovsky, N. Circannual cycles in golden-mantled ground squirrels: Experiments with food deprivation and effects of temperature on periodicity. *J. Comp. Physiol.* 136, 355–360 (1980).
65. Mrosovsky, N. Thermal effects on the periodicity, phasing and persistence of circannual cycles. In *Living in the Cold* (eds Heller, H. C. et al.) 403–410 (Elsevier, New York, 1986).
66. Mrosovsky, N. Circannual cycles in golden-mantled ground squirrels: fall and spring cold pulses. *J. Comp. Physiol.* 167, 683–689 (1990).
67. Canguilhem, B., Schieber, J. P. & Koch, A. Circannual weight rhythm of the European hamster (Cricetus cricetus). Respective influence of the photoperiod and external temperature during its course. *Arch. Sci. Physiol.* 27, 67–90 (1973).
68. Jallageas, M. & Assenmacher, I. External factors controlling annual testosterone and thyroxine cycles in the edible dormouse Glis glis. *Comp. Biochem. Physiol. A Physiol.* 77, 161–167 (1984).
69. Touitou, Y., Smolensky, M. H. & Portaluppi, F. Ethics, standards, and procedures of animal and human chronobiology research. *Chronobiol. Int.* 23, 1083–1096 (2006).

**Acknowledgements**

The authors are grateful to Hassan II Agronomy and Veterinary Medicine Institute (Rabat, Morocco) and the Institute of Cellular and Integrative Neurosciences (CNRS and University of Strasbourg, France) for supporting this study. The authors are thankful to Dr. David Hicks for correcting the English language of the manuscript.

**Author contributions**

H.F. Performed the experiments; Analyzed and interpreted the data and drafted the manuscript. M.R.A. Analyzed and interpreted the data and drafted the manuscript. M.P., M.O and B.B. Contributed reagents, materials, analysis tools or data and drafted the manuscript. P.P. and E.C. Conceived and designed the experiments; Analyzed and interpreted the data and drafted the manuscript. K.E. Conceived and designed the experiments; Analyzed and interpreted the data and drafted the manuscript.

**Funding**

This study was funded by Hassan II Institute of Agronomy and Veterinary Medicine of Rabat-Morocco and the International Foundation for Science (IFS: Research Grant Agreement No. I-3-B-6082-1).

**Competing interests**

The authors declare no competing interests.

**Additional information**

**Supplementary information** is available for this paper at https://doi.org/10.1038/s41598-020-76535-y.

**Correspondence** and requests for materials should be addressed to K.E.A.

**Reprints and permissions information** is available at www.nature.com/reprints.

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s) 2020