Heliyon

Received:
6 June 2018
Revised:
19 November 2018
Accepted:
23 November 2018

Cite as: H. Farsi, D. Harti, M. R. Achaâban, M. Piro, M. Ouassat, E. Challet, P. Pévet, K. El Allali. Validation of locomotion scoring as a new and inexpensive technique to record circadian locomotor activity in large mammals. Heliyon 4 (2018) e00980. doi: 10.1016/j.heliyon.2018.e00980

Validation of locomotion scoring as a new and inexpensive technique to record circadian locomotor activity in large mammals

H. Farsi [a], **D. Harti** [a], **M. R. Achaâban** [a], **M. Piro** [b], **M. Ouassat** [a], **E. Challet** [c], **P. Pévet** [c], **K. El Allali** [a,*]

[a] *Comparative Anatomy Unit, Department of Biological and Pharmaceutical Veterinary Sciences, Hassan II Agronomy and Veterinary Institute, BP: 6202, Rabat-Instituts, 10101, Rabat, Morocco*

[b] *Medicine and Surgical Unit of Domestic Animals, Department of Medicine, Surgery and Reproduction, Hassan II Agronomy and Veterinary Institute, BP: 6202, Rabat-Instituts, 10101, Rabat, Morocco*

[c] *Institute for Cellular and Integrative Neurosciences, CNRS and University of Strasbourg, 5 Rue Blaise Pascal, 67000, Strasbourg, France*

[*] Corresponding author.

E-mail address: khalid_elallali@yahoo.fr (K. El Allali).

Abstract

Background: The locomotor activity (LA) rhythm, widely studied in rodents, has not been fully investigated in large mammals. This is due to the high cost and the brittleness of the required devices. Alternatively, the locomotion scoring method (SM), consisting of attribution of a score to various levels of activity would be a consistent method to assess the circadian LA rhythm in such species.
New method: To test this, a SM with a score ranging from 0 to 5 has been developed and used in two domestic large mammals, the camel and the goat. One minute interval scoring was performed using visual screening and monitoring of infra-red camera recording videos and carried out by two evaluators.
Results: The SM provides a clear daily LA rhythm that has been validated using an automate device, the Actiwatch-Mini. The obtained curves and actograms were indeed highly similar to those acquired from the Actiwatch-Mini. Moreover,

https://doi.org/10.1016/j.heliyon.2018.e00980

there were no statistical differences in the period and acrophase. The period was exactly of 24.0h and the acrophases occurred at 12h05 ± 00h03 and 12h14 ± 00h07 for the camel and at 13h13 ± 00h09 and 12h57 ± 00h09 for the goat using SM and Actiwatch-Mini respectively.

Comparison with existing methods: Compared to the automatic system, the SM is inexpensive and has the advantage of describing all types of performed movements.

Conclusions: The new developed SM is highly reliable and sufficiently accurate to assess conveniently the LA rhythm and specific behaviors in large mammals. This opens new perspectives to study chronobiology in animal models of desert, tropical and equatorial zones.

Keywords: Neuroscience, Physiology

1. Introduction

In mammals, the master circadian clock, located in the hypothalamic suprachiasmatic nuclei (SCN), generates self-sustained oscillations and controls peripheral rhythms by neural and hormonal pathways (Dibner et al., 2010; Pévet, 2014). These rhythmic processes are modulated by environmental cues such light-dark cycle (LD-cycle), food and/or ambient temperature (Ta) (Meijer and Rietveld, 1989; Challet et al., 1997; Berson et al., 2002; Carneiro and Araujo, 2012; Challet, 2013; El Allali et al., 2013). The SCN lesion induces a loss of rest-activity rhythm; while brain graft of fetal SCN cells restores it (Moore and Eichler, 1972; Stephan and Zucker, 1972; Lehman et al., 1987). The locomotor activity (LA) rhythm, one of the most studied circadian rhythm (Hut et al., 1999; Refinetti, 2006; Verwey et al., 2013), is widely investigated in laboratory animals but not so much in large mammals (Bertolucci et al., 2008; Piccione et al., 2010).

Several systems to quantify the daily rhythm of LA have been developed in laboratory animals including running wheel system, infrared system, video-based tracking systems and ultrasonic system (Vatine et al., 1998; Hut et al., 1999; LeGates and Altimus, 2011). In large mammals, piezo-accelerometer systems of actiwatch devices have been developed and successfully used to record LA rhythm for several days in goat, sheep, horse, oryx and elephant (Piccione et al., 2008a, 2008b, 2010; Hetem et al., 2012; Davimes et al., 2017; Gravett et al., 2017). However, these device systems are high costing and easily breakable. Indeed, the unpredictable behavior and bad temper of such large and wild animals (Lindenfors and Tullberg, 2011), may damages the recording devices, making their use a real challenge, especially when recording is carried for a long period, both in wild and captivity conditions.

The search for alternate, inexpensive and reliable technique to study the daily/circadian rhythm of LA in large animals especially in desert, tropical and equatorial zones becomes, nowadays, a real challenge. Meanwhile, the locomotion scoring technique,

https://doi.org/10.1016/j.heliyon.2018.e00980

based on the attribution of a score to different levels of activity is largely used to study the behavior and to evaluate motility, locomotion ability and health states (Kaler et al., 2009; Shigyo et al., 2014; Singh et al., 2014; Angell et al., 2015; Schlageter-Tello et al., 2015). For example, in studies of spinal cord injury in rats and mice, this scoring method constitutes a reliable approach for monitoring LA (Basso et al., 1995, 1996; Scheff et al., 2002; Martinez et al., 2009). To our knowledge, the locomotion scoring has not been used yet to measure the circadian or daily rhythm of LA. This technique could be a reliable way to measure the LA rhythm in large mammals when applied to continuous time scale. To achieve this goal, we recorded the daily rhythm of LA using a locomotor scoring method in two domestic large mammals, the camel (*Camelus dromedarius*) and the goat (*Capra hircus*). This involves an analysis of animal's video-recordings using a score-scale system to evaluate all performed movements. To validate this method, the obtained rhythm data were compared to simultaneously recorded data using an actiwatch device.

2. Material and methods

2.1. Animals and housing conditions

All animal procedures comply with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. There were no animal sacrifices to carry out this study. The work was conducted by using non-invasive techniques based on the observation of the activity of animals through a video-recording. The study was in conformation with the Hassan IInd Agronomy and Veterinary Institute of Rabat and the Moroccan Ministry of Agriculture recommendations which are in accordance with international ethical standards (European Union Directive 2010/63/EU) legislation (Touitou et al., 2006).

The study was conducted at different periods of the year starting from March 2014 to December 2017. Seven local adult female camels (*Camelus dromedarius*) and seven local male goats (*Capra hircus*) originated from the south of Morocco were separately used. The age and weight (mean ± Standard Error Mean (SEM)) were respectively for camel 8.6 ± 1.8 years and 550 ± 42 kg and for goats, 1.5 ± 0.3 years and 28 ± 5 kg. Experiments were conducted at the Hassan IInd Agronomy and Veterinary Institute of Rabat-Morocco (Latitude: 34°00′47″N; Longitude: 6°49′57″O). All animals were kept free and housed individually in outdoor enclosures (10 m × 8 m) under natural environmental conditions. Food was offered twice daily at 10h00 am and 05h00 pm with free access to water.

2.2. Video recording

Video-recordings using a high-resolution camera with infrared emitters (Samsung SCO-2080R®) were used for monitoring of the day/night's activity. The camera

https://doi.org/10.1016/j.heliyon.2018.e00980

was connected to a Digital Video Recorder (DVR) system (Provision ISR®, model: SA-4100HDX+) with a large storage capacity of 1 Terabyte. The recorded videos were transferred to an external storage and viewed on a computer screen for analysis. The "Motion Det" camera's function which offers the monitoring of movements only when they occur was disabled because of a mismatching between the real movement and the on/off of the function. Thus, the study was conducted by using continuous recording mode.

2.3. The locomotor activity scoring

The daily rhythm of LA was measured separately in each animal for a time set of 1 minute during a total period of 10 days, considered as the minimal recording period for accurate analysis in circadian rhythms (Refinetti et al., 2007). The recorded video sequences were visually analyzed on a computer screen. Each activity act was assigned a score for one minute interval. Before establishing a scoring scale, repeated preliminary observations were required to define the major states of movements (motionless, sitting, standing...) for each species. The final established scoring scale for both camel and goat included six scores (0−5) and was as follows:

- **0**: Animal on a sitting position and not moving.
- **1**: Animal on a sitting position and moving lightly its limb under the body.
- **2**: Animal on standing position without movements.
- **3**: Animal on standing position moving his limbs but without locomotion.
- **4**: Animal walking and exploring the environment.
- **5**: Animal in intense locomotor activity or agitated.

To analyze quickly the different segments of recording videos, acceleration mode on the video-screen was used according to the intensity of the animal's activity and the time spent by the animal in the same position/score. This allowed scoring 24h-activity in a reasonable interval-time. Total time spent for video-recording analysis was repeatedly calculated for every 24h sequences and separately for goat and camel. It was presented as the average (mean ± SEM) of two evaluators.

2.4. Validation of locomotor activity scoring method

2.4.1. Reliability and repeatability of the method

The scores assignment was performed by two evaluators well-trained in behavioral scoring. The intra-and inter-assays and the inter-evaluators coefficients of variations (CV) were calculated to examine the accuracy, reliability and stability of the data obtained by the scoring method. The CVs calculation was carried out, separately for the camel and the goat, as follows:

https://doi.org/10.1016/j.heliyon.2018.e00980

- The intra-assay CV was determined as the variation within the obtained scores data of each one minute set from one analysis assay. Each one minute sample of a 24 hours video-recording sequence was scored 5 times in the same analysis by the same evaluator and during the same day. CV was calculated for each sample and then the average of the individual CVs is denoted as intra-assay CV.
- The inter-assay CV, for each evaluator, was calculated as the variation of scoring data of each one minute set obtained from repeated analysis assays. Each one minute sample of the 24 hours video-recording sequences was scored 3 times in the same analysis assay constituting a triplicate by one assay. This analysis was repeated 4 times at interval of 1 week to obtain a total of 5 assays. CV was calculated for each sample/assay and then averaged to monitor the accuracy of the results between the five assays per evaluator.
- The inter-evaluators CV was determined to assess the stability of the obtained data between the two evaluators. Each one minute sample of five continuous 24h video-recording sequences (total of five days of recording) was simultaneously scored, in a blinded way, by the two evaluators. CV between the two evaluators was calculated for each sample/1 minute and then averaged for each assay (day) and finally averaged for the whole five days.

2.4.2. Validation of the obtained rhythm

To validate the LA rhythm obtained from the scoring method, the activity of the same animals was simultaneously recorded by the Actiwatch-Mini® device (Cambridge Neurotechnology Ltd, Cambridge, UK) for a period of 10 days and for all animals. It is a small device (24 mm × 7 mm) that is commonly used to record rest-activity circadian rhythm. It is also used to record LA and all conscious and unconscious movements such as feeding, drinking, walking, grooming and rumination (Piccione et al., 2010). This device is an actigraphy-based data logger which automatically measures the total activity of a wide range of species including large animals. It uses a piezo-electric accelerometer to record the integration of intensity, amount, and duration of movement. The corresponding voltage produced by movements is converted and stored in the memory unit of the Actiwatch-Mini. Connection to the computer via the Actiwatch reader allows to program or to download data from the device.

To record simultaneously the LA rhythm, the Actiwatch-Mini (Fig. 1A) was programmed using its USB reader (Fig. 1B) and protected in a small box then securely appended to the lateral side of the forearm both in the camel (Fig. 1C) and the goat (Fig. 1D). As for the locomotor scoring technique, the recording of LA was set to an interval of one minute.

https://doi.org/10.1016/j.heliyon.2018.e00980

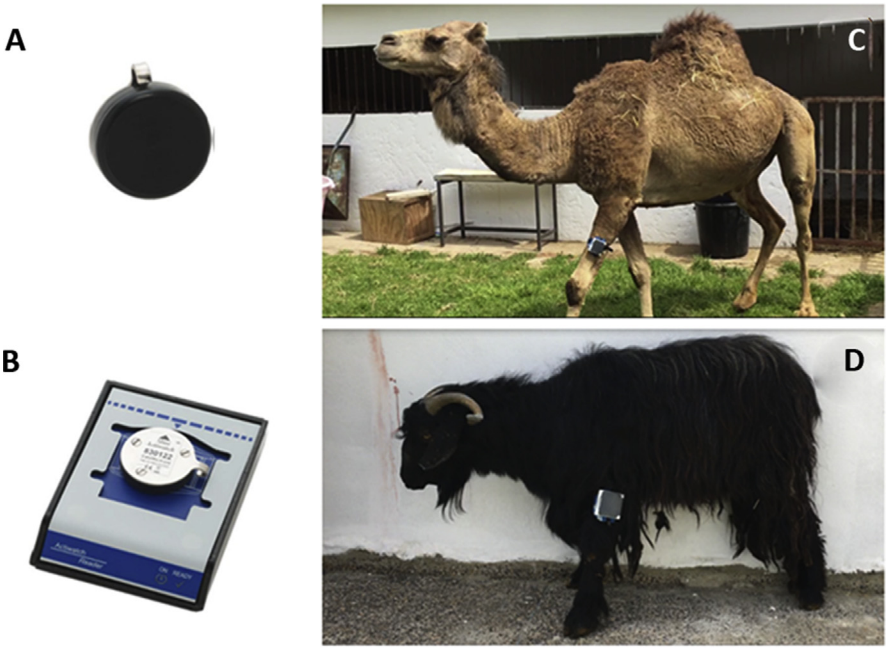

Fig. 1. Images of the piezo electric system used for the validation of the locomotor scoring method of LA rhythm. Actiwatch-Mini® device and its USB reader (A): the device and, (B): its USB reader, (C) and (D): Placement of the device on the lateral side of the forearm of the camel and of the goat, respectively.

2.5. Data analysis

The LA curves were plotted using Sigma-Plot software (Sigmaplot v12.0, Systat, Chicago, IL). Actograms were produced using the ACTOGRAM PLOTTER® software (Refinetti R, Circadian Rhythm Laboratory, University of South Carolina, http://www.circadian.org/softwar.html).

Parameters of LA daily rhythm, including the period, the acrophase, the mesor, the amplitude and the robustness, were calculated both in camels and goats using nonlinear least squares regression, with the help of Cosinor, Acro and LSP software (Refinetti R, Circadian Rhythm Laboratory, University of South Carolina, http://www.circadian.org/softwar.html). Cosinor analysis was performed according to the equation:

$$f = y0 + a^{*}cos[2\pi^{*}(t - \varphi)/\tau]$$

Where f is the LA at time t, $y0$ is the mesor, a is the amplitude, φ is acrophase and τ the period (fixed to 24.0 h). For each parameter, a confidence interval of 95% probability is given. Similarly, the degree of significance of the regression is calculated.

The robustness has been largely used in the circadian literature to describe how regular a circadian rhythm is (Refinetti et al., 1994; Refinetti, 2004a). In the present study, the robustness of the LA rhythm was calculated using cosinor rhythmometry method as described by Nelson et al. (1979). The rhythm robustness is computed as the percentage of variance accounted for by the rhythm and automatically given by Cosinor software. In our study above 20% was considered as a threshold value indicating a statistically significant rhythm (Piccione et al., 2010).

When cosinor fittings were too approximate, especially for the scoring data, acrophase was concurrently determined from the following exponential mathematical equation (Vivien-Roels et al., 1997; Bothorel et al., 2002) and using scoring averaged data of every 5 min:

$$f = y0 + (ymax/((1 + exp(2.7*(phi1 - x)))*(1 + exp(2.7*(x - phi2)))))$$

The f representing LA variation with time x, $y0$ is the basal level measured during daytime, *ymax* is the amplitude of the diurnal pick of LA. The *phi1* and *phi2* represent the inflection point and the time point at which the 50% of the maximal increase and the 50% of the decline were reached respectively. The duration of the LA peak corresponds to the difference between *phi1* and *phi2*. Since the locomotion scoring and its best fitting waves were in regular square shape, the acrophase was calculated as the half of this duration: *phi2-phi1*/2. To validate this obtained calculation, the acrophases of locomotion scoring was also determined from direct inspection of the average curves (see El Allali et al., 2013).

The Spearman Rank Correlation was used as a statistical procedure to calculate the strength of association by ranks between the distributions of locomotion scoring data and the Actiwatch data. The correlation equal to 1 ($r_s = 1$) indicates a perfect association between the two variables. In order to compare the two methods in equal conditions, the raw data of Actiwatch were divided in six categories using their frequencies from the scoring method and the spearman rank correlation was tested to compare each category to the corresponding score.

In addition, the paired Student t-test was used to evaluate the statistical differences between the locomotion scoring and the Actiwatch data by comparing both the periods and the acrophases. This test was also used to determine the statistical differences in the amount of activity between day and night.

To compare if the beginning and the end of the activity rhythm coincide with the dark-light and light-dark transitions, the onset and offset of activity were calculated and compared with the time of these transitions. The onset represents in minutes the difference between the time of the beginning of activity and the time of the dark-light transition. The offset of activity was calculated in minutes as the difference between the time of the end of activity and the time of the light-dark transition. The two way ANOVA was used to determine the statistically differences in the equality of means for 10 consecutive days of the onset and the offset of the camel and goat, respectively and then the Student test of conformity was used to verify the statistical differences between the onset and the offset of LA rhythm with the dark-light and light-dark transitions, respectively.

Differences between the total numbers of attribution of each LA scoring (Percentage of different locomotor activity levels) were compared for day and night in both species using Repeated Measures ANOVA.

All the statistical analyses were carried out using the Sigma-plot software. A $p \leq 0.05$ was considered statistically significant in all statistical tests used including the mathematical regression.

3. Results

3.1. Existence of a daily LA rhythm using locomotion scoring method

Under natural environmental conditions, the used scoring method revealed a clear daily rhythm of LA both in camel and goat (Figs. 2, 3, and 4). It should be considered that in both species, the represented Figs. 2, 3, and 4 show one individual locomotor activity as representative examples. The rhythm follows the LD-cycles as its onset and offset coincide respectively with the dark-light and light-dark transitions. During the day, both species alternate ($P \leq 0.05$) between score “3” and score “4” (Fig. 5). Score “1” and “2” were also frequently observed during the day in the goat. During the night, the sitting position without movements (scores “0”) was the dominant behavior ($P \leq 0.05$) in both species (Fig. 5) while the sitting position with

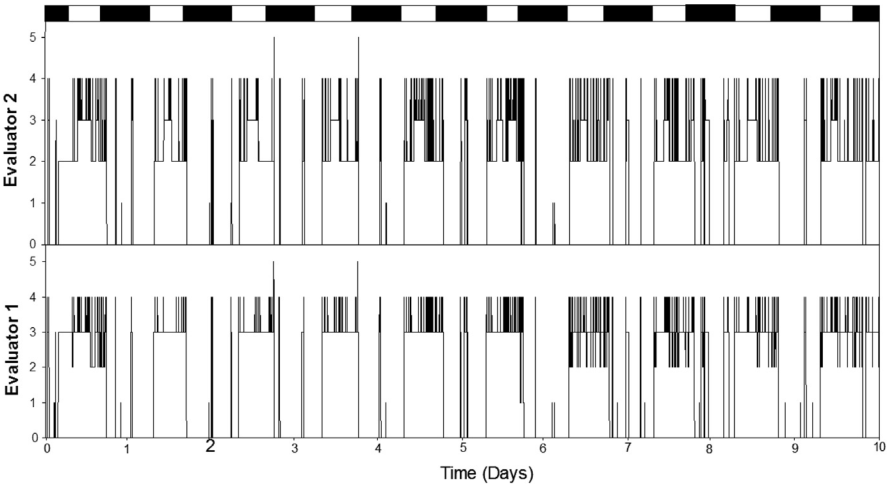

Fig. 2. Actograms of the locomotor activity of a camel under natural conditions during 10 consecutive days, recorded by the scoring method ranging from 0 to 5 made by the evaluator 1 (Scoring 1) and the evaluator 2. Data represents one individual representative camel. Black line indicates the locomotor activity rhythm every minute. The black and white rectangles at the top of the figure correspond to the succession of night (natural dark phase) and day (natural light phase).

https://doi.org/10.1016/j.heliyon.2018.e00980

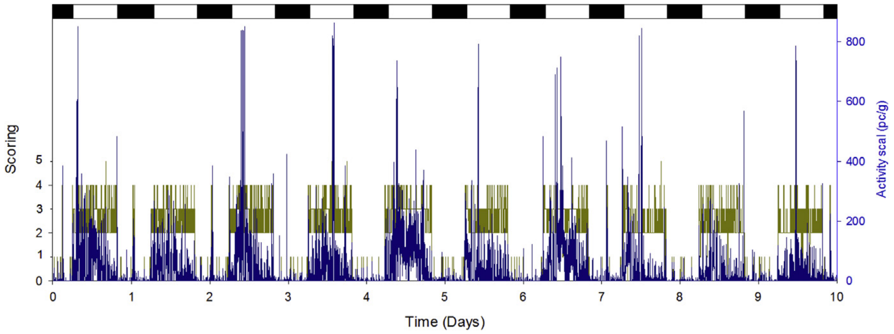

Fig. 3. Superposition view of actograms of the locomotor activity provided by the scoring method (green line) and by the Actiwatch-Mini device (blue line) in one individual representative camel. The black and white rectangles at the top of the figure correspond to the succession of night (natural dark phase) and day (natural light phase).

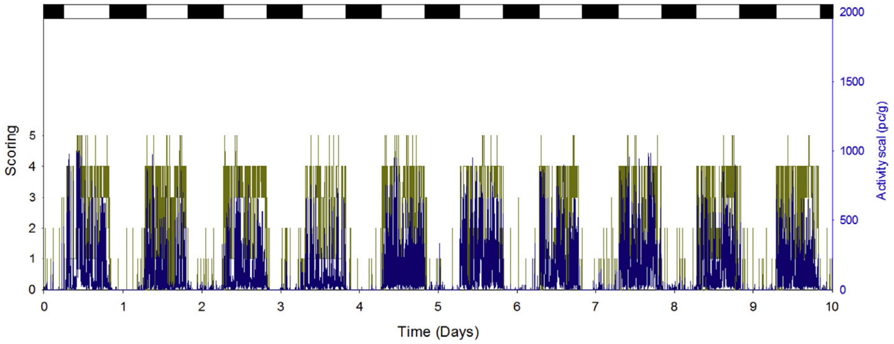

Fig. 4. Superposition view of actograms of the locomotor activity provided by the scoring method (green lines) and by the Actiwatch-Mini (blue line) in one individual representative goat. The black and white rectangles at the top of the figure correspond to the succession of night (natural dark phase) and day (natural light phase).

movements (scores “1”) was sporadically present (Fig. 5). The categories of scores from 2 to 5 were rarely noticed during the night in both species ($P \leq 0.05$).

3.2. Reliability and repeatability of the scoring method

Results of the repeated analysis show that the total time spent for scoring a 24h video-recording averaged 01h00 ± 11 min for camel and 01h21 ± 18 min for goat. The calculated intra-assay CV was ranged between 09.5% and 11.5%, the inter-assay CV between 11% and 12% and the inter-evaluators values CV was less than 09% (Table 1). The obtained low CVs indicate clearly that the scoring method is highly reliable and repeatable, a finding which is also illustrated by the identical LA rhythms obtained by the two evaluators (Fig. 2). A careful examination of the curves showed the existence of few and minor differences in the scoring without affecting the general aspect of the curve.

https://doi.org/10.1016/j.heliyon.2018.e00980

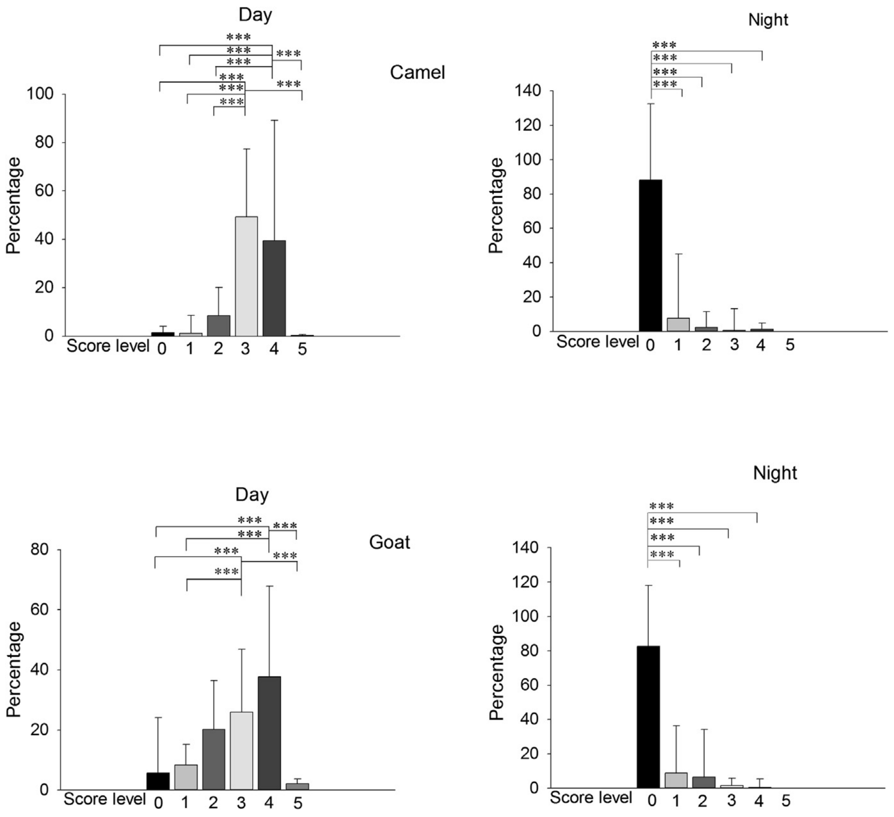

Fig. 5. Percentage of the different LA scores during the day and during the night represented separately for the camel and the goat. The percentage of each score was calculated for each animal for the whole 10 nights and 10 days of experiment and then averaged for all animals of each species. Comparison of the percentage of scores was realized by using Repeated Measures ANOVA. *** P≤0.001 highly significant.

Table 1. The calculated intra-, inter–assay and inter–evaluators coefficients of variation in the goat and the camel.

Evaluators	species	Intra–assay CV	Inter–assay CV	species	Inter–evaluators
Evaluator 1	Camel	9.50%	12.19% ± 0.17	Camel	8.45% ± 0.39
	Goat	10.78%	12.43% ± 0.22		
Evaluator 2	Camel	10.23%	11.45% ± 0.37	Goat	8.89% ± 0.39
	Goat	11.48%	12.14% ± 0.24		

CV: Coefficient of variance %.

3.3. Validation of the data

The visual assessment of the superimposed LA rhythm's curves of the locomotion scoring method and the Actiwatch-Mini® device (Figs. 3 and 4) shows identical pattern of LA in both species. Data were also plotted as actograms (Fig. 6) which show individual representative locomotor activity examples from camel and goat.

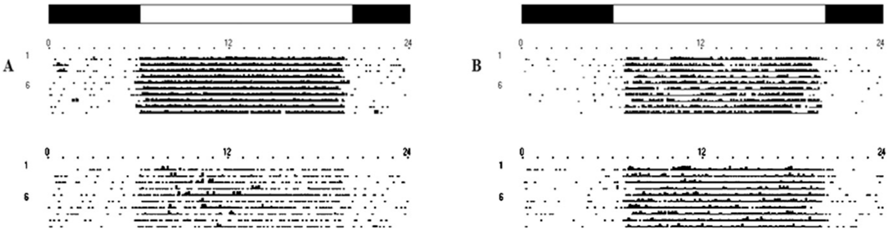

Fig. 6. Actograms of the locomotor activity in one individual representative camel (A) and in one individual representative goat (B) during 10 days obtained by the ACTOGRAM PLOTTER software. The scoring method data are presented at the top and those of Actiwatch device at the bottom. Each line corresponds to 24h of activity starting at 00h00 and ending at 24h00. The lines and black points indicate the existence of a locomotor activity: the scores of 1−5 induce respectively an increase of the thickness of the lines. White or on-line vacuum shows no activity thus representing a score 0. The black and white rectangles at the top of the figure correspond to the night (natural dark phase) and day (natural light phase).

Comparison of the two methods confirms the existence of high level of similarities of the two rhythms. Furthermore, the two rhythms displayed exactly the same relationship with the LD-cycles transitions (Fig. 7). Indeed, the onset and offset of the activity rhythm coincides exactly with these transitions. The two way ANOVA shows non-statistical differences ($P \geq 0.05$) between the different data of the onset and the offset indicating a significant equality of the means. In addition, The Student test of conformity indicates that for all the 10 consecutive days of the experiment, there are no significant differences ($P \geq 0.05$) when comparing the time of the onset and the offset of LA rhythm with the transition moments of the dark-light and light-dark, respectively.

The Spearman Rank Correlation test demonstrates the existence of a strong correlation between the two rhythms obtained by using simultaneously the locomotion scoring method and the Actiwatch device. The coefficient of variation r_s was equal to 0.73 ± 0.01 in camel and 0.74 ± 0.01 in goat. The pairs of the two variables, showing a very highly significant positive correlation ($P \leq 0.0001$) in all animals, indicate that the data profiles conjointly increase and decrease.

The paired *t-test* shows the presence of exactly the same and significant period of 24.0 h ($P \leq 0.0001$) for the rhythms of both scoring method and Actiwatch-Mini device.

The mathematical regression using Cosinor method shows an acrophase statistically different ($P \leq 0.001$) from those calculated using exponential regression and manual determination ($P \leq 0.001$) for scoring method, and also from that calculated by Cosinor for Actiwatch mini's data (Table 2). This difference between scoring's Cosinor acrophase with all other calculated acrophases is probably due to the fact that the equation of Cosinor method does not allow a precise fitting of the scoring data waves that were in regular square shape in contrast to the sinusoidal forms time series as in

https://doi.org/10.1016/j.heliyon.2018.e00980

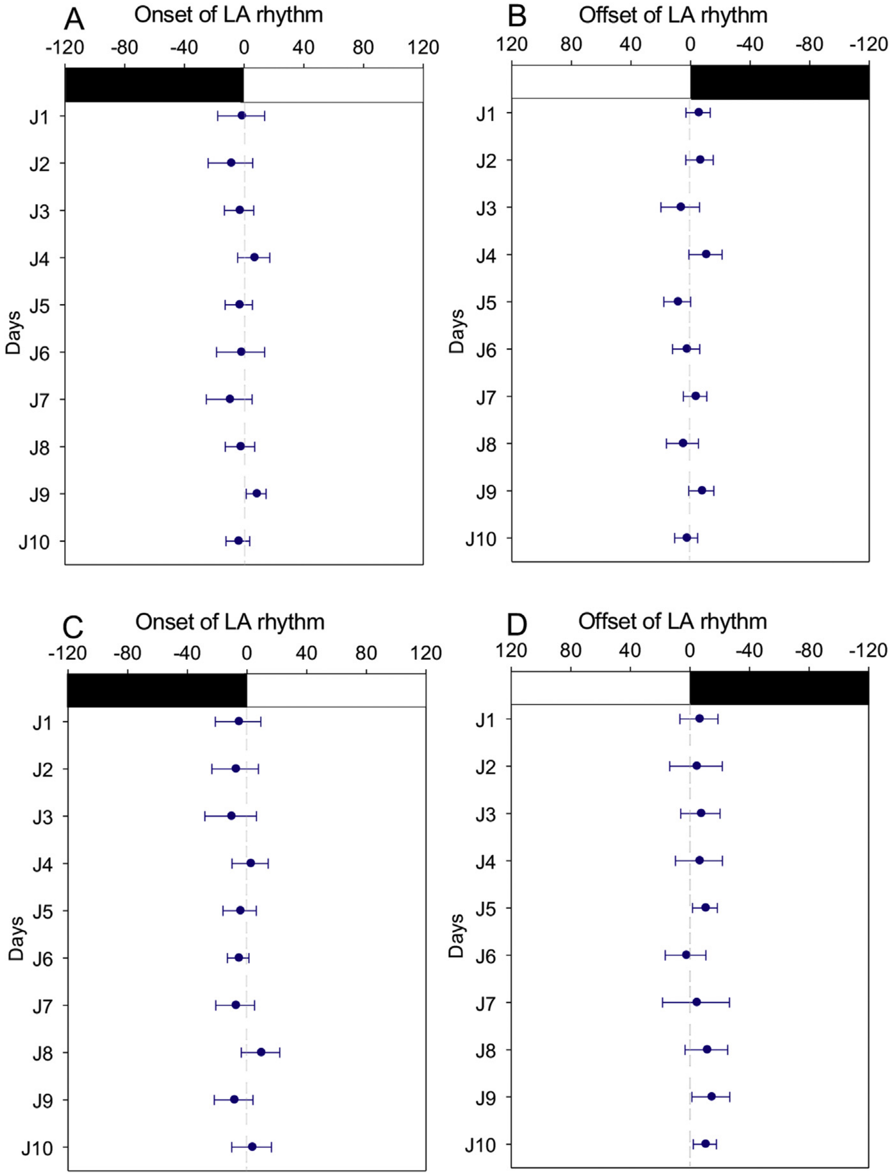

Fig. 7. Means (±SEM) of the onset and the offset of the LA rhythm (dots with error bars) correlated to the dark-light and light-dark transitions in the camel (A) & (B), and in the goat (C) & (D) during 10 consecutive days. The onset of activity was calculated as the difference between the time of the beginning of activity with the time of the dark-light transition. The offset of activity was calculated as the difference between the time of the end of activity with the time of the light-dark transition. The transitions of dark-light and light-dark are considered respectively as the reference with the given time 0 minutes. These transitions in natural environment are gradual and last 20–25 minutes. The time of transition in the present figure correspond to the official time of sunrise and sunset provided by the Regional Direction of Meteorology of Rabat-Morocco. The black and white rectangles at the top of each figure correspond to the natural dark phase of the night and the natural light phase of day.

https://doi.org/10.1016/j.heliyon.2018.e00980

Table 2. Comparison between the acrophase of scoring method, calculated manually, and Actiwatch-Mini in the camel and goat.

Animals	locomotion scoring			Actiwatch-Mini
	Acrophase calculated by the exponential regression equation†	Acrophase calculated manually‡	Acrophase calculated by Cosinor§	Acrophase calculated by Cosinor#
Camel 1	11h56	12h00	13h33***	12h06
Camel 2	12h08	12h09	13h50***	12h02
Camel 3	12h01	12h06	12h54***	12h03
Camel 4	12h08	12h11	13h18***	12h14
Camel 5	12h14	12h10	13h05***	12h22
Camel 6	11h58	12h26	13h08***	12h53
Camel 7	12h16	12h10	13h22***	12h00
Mean	12h05 ± 00h03	12h10 ± 00h03	13h18 ± 00h07	12h14 ± 00h07
Goat 1	13h56	13h47	14h10***	13h39
Goat 2	13h44	13h52	14h24***	12h42
Goat 3	12h55	12h48	13h57***	13h08
Goat 4	13h16	13h09	13h44***	12h19
Goat 5	13h27	13h12	14h59***	13h02
Goat 6	12h42	12h39	13h45***	12h59
Goat 7	13h33	12h35	14h08***	12h50
Mean	13h21 ± 00h09	13h08 ± 00h12	14h09 ± 00h10	12h57 ± 00h09

Note: The comparisons of the †, ‡, and # with each other indicate that there is no significant difference $P \geq 0.05$. The comparison of the § with †, ‡, and # indicate that $P \leq 0.001$ is strongly significant. Symbols “***” in acrophases of the column § indicate strong significance in comparison to acrophases of columns †, ‡, and #.

the Actiwatch data (Fig. 8). Indeed, the application of the exponential regression allowed better fittings of scoring method data curves time series in square shape (Fig. 8). This was confirmed by the absence of significant differences between the acrophase of the scoring method provided by the exponential equation and the acrophase of the Actiwatch data calculated by the Cosinor. In addition, there were no statistical differences between this acrophase of the exponential equation with that of the scoring method data calculated manually.

The paired *t-test* did not detect any statistical differences ($P \geq 0.05$) between acrophases of LA scoring calculated manually or by the exponential regression and those of actiwatch mini calculated by Cosinor regression (Table 2). Thus, both LA scoring and actiwatch mini showed the same acrophases with only 10−15 minutes of no significant differences. In fact, the acrophase in the camel was at 12h05 ± 00h03 for the locomotion scoring rhythm and at 12h14 ± 00h07 for the Actiwatch-Mini rhythm. The corresponding values in the goat were respectively 13h13 ± 00h09 and 12h57 ± 00h09.

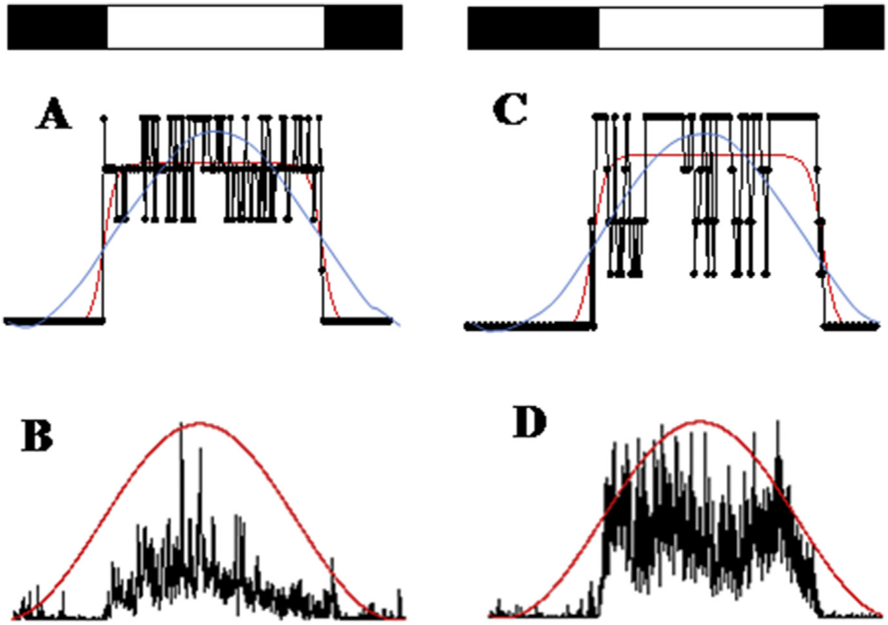

Fig. 8. Regressions, performed on the 24h-educed rhythm of locomotor activity of 10 days on the locomotion scoring data ($p < 0.0001$) and the Actiwatch-Mini data ($p < 0.001$). The regression represents superimposition of the 24h curves of scoring method (A) and Actiwatch Mini device (B) in the camel and respectively (C) and (D) in the goat. The red curves are the best fitting curves of regressions which are the exponential regression for locomotion scoring (red in A and C) and the Cosinor for actiwatch data (red in B and D) the blue curves in A and C represent the curves of regression of Cosinor method that does not fit good with locomotion scoring data. Black and white barres denote of respectively the day and night phases.

The mesor and the amplitude of the LA rhythm in the camel, obtained from the scoring method, were respectively 1.85 ± 0.25 and 2.72 ± 0.21; while those acquired by the Actiwatch device were 315 ± 56.37 and 483.5 ± 49.70 respectively ($P \leq 0.05$). In the goat, the corresponding values were 1.64 ± 0.30 and 2.5 ± 0.33 for the scoring method and 406.14 ± 103.42 and 583.33 ± 49.52 for the Actiwatch device respectively ($P \leq 0.05$). In the present study, comparisons of the mesor and the amplitude between the two techniques could not be undertaken because the units and scales are not the same.

The LA rhythm robustness (denoting its regularity rate) was of 61.8 ± 4.4% in the camel and 45.0 ± 3.4% in the goat for the scoring method, while that of Actiwatch device was 50.3 ± 2.9% and 49.7 ± 2.5% respectively in the camel and the goat.

3.4. Diurnality of the LA rhythm

The visual assessment of the actograms (Fig. 6), obtained from the scoring method and the Actiwatch-Mini, revealed that, both in the camel and the goat, the LA rhythm shows high values during the day, starting with the beginning of the light phase at 06h52am and extends to its end at 08h00pm. Then, the activity declines progressively all over the night. As mentioned above, the rhythm follows the light-dark cycle as its onset and the offset coincide respectively with the dark-light and light-dark transitions in both species (Fig. 7). The total numbers of attribution of each LA score were as reported above significantly higher ($P \leq 0.05$) during the day for all the scores denoting activity: 3, 4 and 5 (Fig. 5).

The mean amounts of total activity (Fig. 9) recorded by the Actiwatch were significantly higher ($P \leq 0.05$) during the day (45026.00 ± 3826.42 for the camels and 119586.14 ± 2058.42 for the goat) compared to those during the night (7083.4827 ± 445.7662 for the camels and 12811.4265 ± 410.6062 for the goat). The scoring method showed the same results with significantly ($P \leq 0.05$) high amount of total activity during the day. The related daily mean amount of total activity was 1735.28 ± 151.57 for the camel and 2196.24 ± 44.58 for the goats. While, during the night, the values dropped to reach 116.33 ± 23.84 for the camel and 166.61 ± 40.70 for the goat. These results emphasize the diurnal nature of these two species, which can be further confirmed by the corresponding acrophases occurring in the middle of the day (Table 2 & Fig. 8).

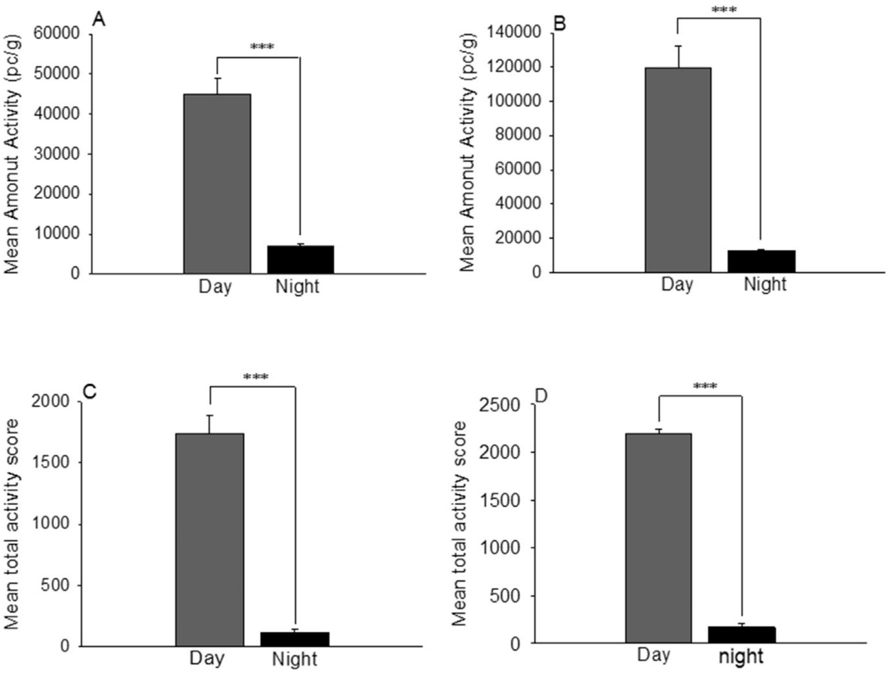

Fig. 9. Amount of the mean total activity during the day and night recorded by the Actiwatch-Mini in the camel (A), in the goat (B) and recorded by the scoring method in the camel (C) and in the goat (D). For the scoring method, the total activity was calculated for each animal as the cumulative of the scores 3, 4 and 5 summed for all nights then all days of experiment and then averaged for the 7 animals of each species. The total activity for the Actiwatch was calculated using Actiwatch Activity Analysis 7.46 (Cambridge Neurotechnology Ltd, UK). Comparison of the amount of the mean total activity during the day and night was realized using the paired Student t-test. *** $P \leq 0.001$ highly significant.

https://doi.org/10.1016/j.heliyon.2018.e00980

4. Discussion

In the present study, a scoring method describing the locomotor activity rhythm and its intensity in large animals has been developed. This new technique provides a continuous system for monitoring the LA and, therefore, is one of the strengths of the study. It corroborates the existence of a daily rhythm of LA in the goat, previously demonstrated in studies using automatic devices recording (Bertolucci et al., 2008; Piccione et al., 2005, 2008b, 2010). In the camel, to our best knowledge, the present use of a locomotion scoring method provides the first evidence of the existence of LA rhythm in this species.

Usually in behavioral investigation, locomotion scoring method uses 5 minutes as a standard time set for scoring (Shigyo et al., 2014; Singh et al., 2014). The daily rhythm of LA was measured separately in each animal for a time set of 1 minute during a total period of 10 days. We believe that this time set allows a highly sensitive and accurate description of LA both in the camel and the goat, which is confirmed by the obtained results. Likewise, the calculated CVs (lower than 20%) indicate clearly that the scoring method is highly reliable and repeatable. It is well known that the CVs should be less than 20% to have a high stability of the data (Forkman, 2009). The use of scoring method in some behavioral studies has shown similar CVs (Shigyo et al., 2014). The fact that the two evaluators scoring obtained identical rhythms exhibits a low inter-evaluators CV. On the other hand, the obtained LA rhythm has been validated by using an accurate accelerometer device (the Actiwatch-Mini®), previously used in different species including the goat, sheep, horse, rabbit, cow and dog (Bertolucci et al., 2008; Giannetto et al., 2010, 2018; Piccione et al., 2010, 2013). The Spearman Rank Correlation test also demonstrates the existence of a strong correlation between the two methods and the paired *t-test* confirmed the existence of the same period and acrophase. Obviously, the used scoring method allows a recording of the LA, as a daily rhythm, similar to that obtained by automatic devices but, in addition, emphasizes all types of activity. The attributed scores are simple, accurate and easy to use. In fact, this technique has been developed after long successive preliminary examinations of recording videos of both species. Once the scores attributed to the different activities exhibited by the animals were standardized, a well-trained observer was needed to analyze the recorded videos. The total time spent for scoring a 24h video-recording was around 01hour. Experiments of chronobiology are usually conducted for a period of at least 10 days (Piccione et al., 2003; Refinetti, 2004b). Thus the video-scoring of such period would take 10 hours. The time spent for video-recording analysis is still in agreement with the most moderate time-consuming laboratory techniques used in the field. Development of an algorithm like that existing for specific behavioral studies in goat (Endo et al., 2016) and pig (Lind et al., 2005) could offer further opportunities to reduce the time spent in analyzing the recorded videos. Nevertheless, the present scoring technique is adequate

https://doi.org/10.1016/j.heliyon.2018.e00980

to record accurately the daily LA rhythm in large animals and can perceive other behavioral phenomena that cannot be distinguished by an algorithm. The Actiwatch measures acceleration and is more accurate in detection of LA than the scoring method. However, the later has the advantage to detect all kinds of movements performed by the animal during 24h; while, the automatic devices are not able to do so, since only conscious and unconscious movements are considered as locomotor activity (total LA) (Hetem et al., 2012; Giannetto et al., 2010; Piccione et al., 2010). For example, it is not possible to distinguish, from the data of the Actiwatch device if the animal moves in standing position (score 3), or walks (score 4) or displays an agitated state (score 5). Furthermore, the absence of activity within the Actiwatch-Mini actograms may indicate a sitting position without movements (score 0) or just a standing position without movements (score 2).

In addition to the conception of a reliable technique for recording daily LA rhythm in large animals, the present study also emphasizes the diurnal pattern of LA in the camel and the goat, as was also demonstrated in other species (Bertolucci et al., 2008; Piccione et al., 2005, 2008a, 2010; Aube et al., 2017). This observed diurnal pattern is very close to that of diurnal laboratory animals (Garcia-Allegue et al., 1999; Schumann et al., 2005).

5. Conclusions

The developed new technique of scoring needs the involvement of a well-trained evaluator to make use of a scoring scale, from 0 to 5, to describe the presence and the intensity of LA from the recording videos. Furthermore, this technique is not very time-consuming, not expensive, highly reliable and able to record efficiently the LA rhythm in large animals. Thus, it is easy and adequate to be used for chronobiological studies in various laboratories around the world, especially where financial aspects are the most important issues facing the research.

Declarations

Author contribution statement

Hicham Farsi: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Driss Harti: Performed the experiments.

Mohamed Rachid Achaâban, Etienne Challet: Analyzed and interpreted the data; Wrote the paper.

Mohammed Piro, Mohammed Ouassat: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Paul Pévet: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Khalid El Allali: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by the comparative Anatomy Unit and the Medicine and Surgical Unit of the Hassan II Agronomy and Veterinary Institute in Rabat, Morocco.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

Authors thank Professor Mohammed Dehhaoui for the help in statistical procedures.

References

Angell, J.W., Cripps, P.J., Grove-White, D.H., Duncan, J.S., 2015. A practical tool for locomotion scoring in sheep: reliability when used by veterinary surgeons and sheep farmers. Vet. Rec. 176 (20), 521.

Aube, L., Fatnassi, M., Monaco, D., Khorchani, T., Lacalandra, G.M., Hammadi, M., Padalino, B., 2017. Daily rhythms of behavioral and hormonal patterns in male dromedary camels housed in boxes. PeerJ 5, e3074.

Basso, D.M., Beattie, M.S., Bresnahan, J.C., 1995. A sensitive and reliable locomotor rating scale for open field testing in rats. J. Neurotrauma 12 (1), 1−21.

Basso, D.M., Beattie, M.S., Bresnahan, J.C., Anderson, D.K., Faden, A.I., Gruner, J.A., Holford, T.R., Hsu, C.Y., Noble, L.J., Nockels, R., et al., 1996. MASCIS evaluation of open field locomotor scores: effects of experience and teamwork on reliability. Multicenter Animal Spinal Cord Injury Study. J. Neurotrauma 13 (7), 343−359.

Berson, D.M., Dunn, F.A., Takao, M., 2002. Phototransduction by retinal ganglion cells that set the circadian clock. Science 295, 1070−1073.

Bertolucci, C., Giannetto, C., Fazio, F., Piccione, G., 2008. Seasonal variations in daily rhythms of activity in athletic horses. Animal 2 (7), 1055−1060.

https://doi.org/10.1016/j.heliyon.2018.e00980

Bothorel, B., Barassin, S., Saboureau, M., Perreau, S., Vivien-Roels, B., Malan, A., Pévet, P., 2002. In the rat, exogenous melatonin increases the amplitude of pineal melatonin secretion by a direct action on the circadian clock. Eur. J. Neurosci. 16 (6), 1090–1098.

Carneiro, B.T., Araujo, J.F., 2012. Food entrainment: major and recent findings. Front. Behav. Neurosci. 6, 83.

Challet, E., Pévet, P., Vivien-Roels, B., Malan, A., 1997. Phase-advanced daily rhythms of melatonin, body temperature, and locomotor activity in food-restricted rats fed during daytime. J. Biol. Rhythm. 12 (1), 65–79.

Challet, E., 2013. Circadian clocks, food intake, and metabolism. Prog Mol Biol Transl Sci 119, 105–135.

Davimes, J.G., Alagaili, A.N., Bertelsen, M.F., Mohammed, O.B., Hemingway, J., Bennett, N.C., Manger, P.R., Gravett, N., 2017. Temporal niche switching in Arabian oryx (*Oryx leucoryx):* seasonal plasticity of 24h activity patterns in a large desert mammal. Physiol. Behav. 177, 148–154.

Dibner, C., Schibler, U., Albrecht, U., 2010. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annu. Rev. Physiol. 72, 517–549.

El Allali, K., Achaaban, M.R., Bothorel, B., Piro, M., Bouaouda, H., El Allouchi, M., Ouassat, M., Malan, A., Pevet, P., 2013. Entrainment of the circadian clock by daily ambient temperature cycles in the camel (*Camelus dromedarius*). Am. J. Physiol. Regul. Integr. Comp. Physiol. 304 (11), R1044–R1052.

Endo, N., Rahayu, L.P., Arakawa, T., Tanaka, T., 2016. Video tracking analysis of behavioral patterns during estrus in goats. J. Reprod. Dev. 62 (1), 115–119.

Forkman, J., 2009. Estimator and tests for common coefficients of variation in normal distributions. Commun. Stat. Theor. Meth. 38 (2), 233–251.

Garcia-Allegue, R., Lax, P., Madariaga, A.M., Madrid, J.A., 1999. Locomotor and feeding activity rhythms in a light-entrained diurnal rodent, *Octodon degus*. Am. J. Physiol. 277 (2), R523–R531.

Giannetto, C., Casella, S., Caola, G., Piccione, G., 2010. Photic and non-photic entrainment on daily rhythm of locomotor activity in goats. Anim. Sci. J. 81 (1), 122–128.

Giannetto, C., Giudice, E., Acri, G., Fazio, F., Piccione, G., 2018. Interspecies comparison of daily total locomotor activity monitoring in different management conditions. J. Vet. Behav. 23, 97–100, 2018/01/01/.

Gravett, N., Bhagwandin, A., Sutcliffe, R., Landen, K., Chase, M.J., Lyamin, O.I., Siegel, J.M., Manger, P.R., 2017. Inactivity/sleep in two wild free-roaming African elephant matriarchs - does large body size make elephants the shortest mammalian sleepers? PloS One 12 (3), e0171903.

Hetem, R.S., Strauss, W.M., Fick, L.G., Maloney, S.K., Meyer, L.C., Shobrak, M., Fuller, A., Mitchell, D., 2012. Does size matter? Comparison of body temperature and activity of free-living Arabian oryx (*Oryx leucoryx*) and the smaller Arabian sand gazelle (*Gazella subgutturosa marica*) in the Saudi desert. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 182 (3), 437−449.

Hut, R.A., Mrosovsky, N., Daan, S., 1999. Nonphotic entrainment in a diurnal mammal, the European ground squirrel (*Spermophilus citellus*). J. Biol. Rhythm. 14 (5), 409−419.

Kaler, J., Wassink, G.J., Green, L.E., 2009. The inter- and intra-observer reliability of a locomotion scoring scale for sheep. Vet. J. 180 (2), 189−194.

LeGates, T.A., Altimus, C.M., 2011. Measuring circadian and acute light responses in mice using wheel running activity. JoVE 48, 2463.

Lehman, M.N., Silver, R., Gladstone, W.R., Kahn, R.M., Gibson, M., Bittman, E.L., 1987. Circadian rhythmicity restored by neural transplant. Immunocytochemical characterization of the graft and its integration with the host brain. J. Neurosci. 7 (6), 1626−1638.

Lind, N.M., Vinther, M., Hemmingsen, R.P., Hansen, A.K., 2005. Validation of a digital video tracking system for recording pig locomotor behaviour. J. Neurosci. Methods 143 (2), 123−132.

Lindenfors, P., Tullberg, B.S., 2011. Evolutionary aspects of aggression the importance of sexual selection. Adv. Genet. 75, 7−22.

Martinez, M., Brezun, J.M., Bonnier, L., Xerri, C., 2009. A new rating scale for open-field evaluation of behavioral recovery after cervical spinal cord injury in rats. J. Neurotrauma 26 (7), 1043−1053.

Meijer, J.H., Rietveld, W.J., 1989. Neurophysiology of the suprachiasmatic circadian pacemaker in rodents. Physiol. Rev. 69 (3), 671−707.

Moore, R.Y., Eichler, V.B., 1972. Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res. 42 (1), 201−206.

Nelson, W., Tong, Y.L., Lee, J.K., Halberg, F., 1979. Methods for cosinor rhythmometry. Chronobiologia 6, 305−323.

https://doi.org/10.1016/j.heliyon.2018.e00980

Pévet, P., 2014. The internal time-giver role of melatonin. A key for our health. Rev. Neurol. (Paris) 170 (11), 646–652.

Piccione, G., Arfuso, F., Giannetto, C., Faggio, C., Panzera, M., 2013. Effect of housing conditions and owner's schedule on daily total locomotor activity in dogs (*Canis familiaris*). Biol. Rhythm Res. 44, 778–786, 2013/10/01.

Piccione, G., Caola, G., Refinetti, R., 2003. Circadian rhythms of body temperature and liver function in fed and food-deprived goats. Comp. Biochem. Physiol. Mol. Integr. Physiol. 134 (3), 563–572.

Piccione, G., Caola, G., Refinetti, R., 2005. Temporal relationships of 21 physiological variables in horse and sheep. Comp. Biochem. Physiol. Mol. Integr. Physiol. 142 (4), 389–396.

Piccione, G., Giannetto, C., Assenza, A., Fazio, F., Giovanni, C., 2008a. Locomotor activity and serum tryptophan and serotonin in goats: daily rhythm. J. Appl. Biomed. 6 (2), 73–79.

Piccione, G., Giannetto, C., Casella, S., Caola, G., 2008b. Circadian activity rhythm in sheep and goats housed in stable conditions. Folia Biol. 56 (3-4), 133–137.

Piccione, G., Giannetto, C., Casella, S., Caola, G., 2010. Daily locomotor activity in five domestic animals. Anim. Biol. Leiden 60 (1), 15–24.

Refinetti, R., Kaufman, C.M., Menaker, M., 1994. Complete suprachiasmatic lesions eliminate circadian rhythmicity of body temperature and locomotor activity in golden hamsters. J. Comp. Physiol. 175, 223–232.

Refinetti, R., 2004a. Non-stationary time series and the robustness of circadian rhythms. J. Theor. Biol. 227, 571–581.

Refinetti, R., 2004b. Daily activity patterns of a nocturnal and a diurnal rodent in a semi-natural environment. Physiol. Behav. 82 (2-3), 285–294.

Refinetti, R., 2006. Variability of diurnality in laboratory rodents. J. Comp. Physiol. A Neuroethol. Sensory Neural Behav. Physiol. 192 (7), 701–714.

Refinetti, R., Lissen, G.C., Halberg, F., 2007. Procedures for numerical analysis of circadian rhythms. Biol. Rhythm Res. 38 (4), 275–325.

Scheff, S.W., Saucier, D.A., Cain, M.E., 2002. A statistical method for analyzing rating scale data: the BBB locomotor score. J. Neurotrauma 19 (10), 1251–1260.

Schlageter-Tello, A., Bokkers, E.A.M., Koerkamp, P.W.G.G., Van Hertem, T., Viazzi, S., Romanini, C.E.B., Halachmi, I., Bahr, C., Berckmans, D., Lokhorst, K., 2015. Comparison of locomotion scoring for dairy cows by

https://doi.org/10.1016/j.heliyon.2018.e00980

experienced and inexperienced raters using live or video observation methods. Anim. Welf. 24 (1), 69–79.

Schumann, D.M., Cooper, H.M., Hofmeyr, M.D., Bennett, N.C., 2005. Circadian rhythm of locomotor activity in the four-striped field mouse, *Rhabdomys pumilio*: a diurnal African rodent. Physiol. Behav. 85 (3), 231–239.

Shigyo, M., Tanabe, N., Kuboyama, T., Choi, S.H., Tohda, C., 2014. New reliable scoring system, Toyama mouse score, to evaluate locomotor function following spinal cord injury in mice. BMC Res. Notes 7, 332.

Singh, A., Krisa, L., Frederick, K.L., Sandrow-Feinberg, H., Balasubramanian, S., Stackhouse, S.K., Murray, M., Shumsky, J.S., 2014. Forelimb locomotor rating scale for behavioral assessment of recovery after unilateral cervical spinal cord injury in rats. J. Neurosci. Methods 226, 124–131.

Stephan, F.K., Zucker, I., 1972. Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. Proc. Natl. Acad. Sci. U. S. A 69 (6), 1583–1586.

Touitou, Y., Smolensky, M.H., Portaluppi, F., 2006. Ethics, standards and procedures in human and animal research in chronobiology. Chronobiol. Int. 23, 1083–1096.

Vatine, J.J., Ratner, A., Dvorkin, M., Seltzer, Z., 1998. A novel computerized system for analyzing motor and social behavior in groups of animals. J. Neurosci. Methods 85 (1), 1–11.

Verwey, M., Robinson, B., Amir, S., 2013. Recording and analysis of circadian rhythms in running-wheel activity in rodents. JoVE 71 pii: 50186.

Vivien-Roels, B., Pitrosky, B., Zitouni, M., Malan, A., Canguilhem, B., Bonn, D., Pévet, P., 1997. Environmental control of the seasonal variations in the daily pattern of melatonin synthesis in the European hamster, *Cricetus cricetus*. Gen. Comp. Endocrinol. 106, 85–94.