

## Daily rhythm of total activity/rest pattern in small and large domestic animals

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Circadian rhythms represent an inherent property of living organisms that seem to guarantee an optimal functioning of the biological system, with maximum efficiency, performance and welfare.<sup>1</sup> In mammals, a master clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus adjusts the timing of other self-sustained oscillators in the brain and peripheral organs.<sup>2</sup> In most species, the daily light-dark (L/D) cycle is the primary environmental stimulus (Zeitgeber) for the entrainment of the SCN pacemaker. The SCN receives light information from the retina and regulates several physiological processes by synchronizing molecular clockwork mechanisms consisted by a core group of clock genes in each cell.<sup>3</sup> Among all physiological processes, the total locomotor activity (TLA) is one of the most susceptible to the L/D cycle. Light acutely suppresses locomotor activity in nocturnal (night active) animals such as rats and owls but promotes activity in diurnal (day active) animals like dogs and eagles.<sup>4</sup> Since animals have a species-typical organization of activity patterns,<sup>5</sup> the aim of this study was to compare the TLA in small and large domestic animals like rabbits, cats, dogs, goats, sheep, cows, donkeys and horses. Five clinically healthy female subjects from eight different species: rabbits (body weight  $2.5 \pm 0.2$  kg), cats (body weight  $4.5 \pm 0.3$  kg), dogs (body weight  $13.5 \pm 1$  kg), goats (body weight  $40 \pm 2$  kg), sheep (body weight  $45 \pm 2$  kg), cows (body weight  $390 \pm 10$  kg), donkeys (body weight  $395 \pm 20$ ) and horses (body weight  $565 \pm 42$ ) were enrolled in the study with owners consent. Animals were housed under natural photoperiod (March) 12:12 hours L/D cycle (5.30 am sunrise, 5.30 pm sunset) according to specific farm management, except for cats and dogs that lived outdoors. Water was available *ad libitum* and feeding was suitable for each species. Total activity pattern was recorded for 10 days using actigraphy-based data loggers Actiwatch-Mini (Cambridge Neurotechnology Ltd, UK) placed on each animal through collars or halters according to the species. Activity was monitored with a sampling interval of 5 minutes. Total daily amount of activity, amount of activity during the photophase and the scotophase were calculated

using Actiwatch Activity Analysis 5.06 (Cambridge Neurotechnology Ltd, UK). The Cosine peak of a rhythm (the time of the daily peak) was computed by cosinor rhythmometry<sup>6</sup> as implement in the Actiwatch Activity Analysis 5.06 program. The temporal resolution of the locomotor activity data was reduced to 1 h bins by the averaging of all 15 data points within each 1 h bin to apply the statistical analysis. To analyze the locomotor activity a trigonometric statistical model was applied to each time series to statistically describe the periodic phenomenon, by characterizing the main rhythmic parameters according to the single cosinor procedure.<sup>6</sup> Four rhythmic parameters were determined: mean level, amplitude, acrophase (the time at which the peak of a rhythm occurs), and robustness (strength of rhythmicity). For each animal, the mean level of the rhythm was computed as the arithmetic mean of all values in the data set (24 data points). The amplitude of the rhythm was calculated as half the maximum-minimum range of the oscillation, which was computed as the difference between peak and trough. Robustness was computed as the percentage of the maximal score attained by the chi-square periodogram statistic for ideal data sets of comparable size and 24-h periodicity.<sup>7</sup> Two-way analysis of variance (ANOVA) was used for the assessment of effects due to species and days on the daily amount of activity per 24 h. Statistical analysis showed significant differences among domestic species. The highest daily amount of activity was observed during the photophase ( $p < 0.0001$ ) in dogs, sheep, goats, cows, donkeys and horses, and during the scotophase ( $p < 0.0001$ ) in rabbits and cats. Our results show different pattern of locomotor activity in every domestic species (Figure 1), underlining a diurnal pattern of locomotor activity in dogs, goats, sheep, cows, donkeys and horses while rabbits and cats have a main nocturnal pattern. As previously observed by several authors,<sup>8-10</sup> our study confirms that locomotor activity exhibits a robust daily rhythm-

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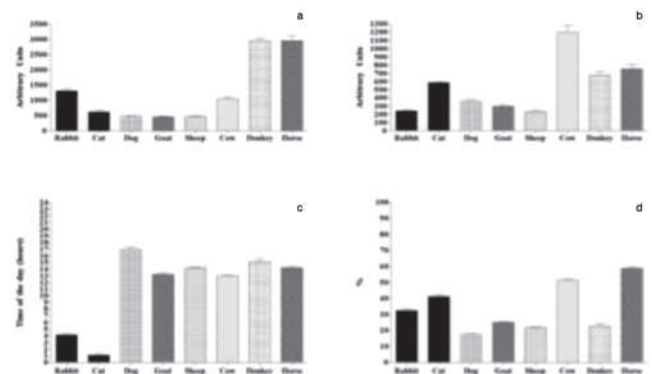


Figure 1. Analysis of rhythm parameters in domestic species: a. Total Locomotor Activity, b. Amplitude, c. Acrophase, d. Robustness.

micity during the photophase in dogs, cows and horses, therefore in these species the rhythm can be poorly affected by external stimuli. On the contrary, other domestic species can spontaneously shift from diurnal to nocturnal activity pattern. Sheep with restricted night time feeding can shift the main bout of activity during the night<sup>8</sup> or cats, that are considered mainly nocturnal, use to loose their rhythm when they live in symbiosis with humans.<sup>11</sup> Therefore, the daily pattern of TLA does not depend only on L/D cycle but it can be affected by several environmental variables including different activities such as feeding, drinking, walking, grooming, playing as well as all conscious and unconscious movements.

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