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RESEARCH ARTICLE

Comparison between circadian motor activity in pony and horse

Comparación de la actividad motora circadiano en ponis y caballos

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ABSTRACT

In this study we investigated the influence of body mass on the daily rhythm of total motor activity in ponies and horses. Six clinically healthy Shetland pony breed with a body mass of 180 ± 30 kg and six healthy horses Sella Italiana breed with a body mass of 530 ± 20 kg were used. Animals were equipped with actigraphy-based data loggers, Actiwatch-Mini® to record total activity. The application of two-way ANOVA showed a highly significant effect of time of day and body mass on motor activity. Cosinor analysis identified the periodic parameters and their acrophases during the two days of monitoring. Mesor and amplitude were statistically higher in horses than in ponies and no statistically significant differences was found about acrophases and robustness. In conclusion we suppose that the difference in the amount of total motor activity, in animals that differ in breed, as pony and horse, may lie in physical differences.

Key words: body mass, daily rhythm, *Equus caballus*, locomotor activity.

RESUMEN

En este estudio hemos investigado la influencia de la masa corporal con respecto al ritmo circadiano de toda la actividad motora en los ponis y los caballos. Han sido utilizados seis sanos Shetland poni con una masa corporal de 180 ± 30 kg y seis sanos caballos biotipo Sella Italiana con una masa corporal de 530 ± 20 kg clínicamente sanos. Los animales han sido equipados con registrador de datos Actiwatch-Mini® para grabar toda la actividad motora. La aplicación de ANOVA dos vías mostró un efecto altamente significante de la hora del día y de la masa corporal sobre la actividad motora. El análisis del Cosinor ha identificado los parámetros periódicos y ellos acrofases en los dos días de seguimiento. Mesor y amplitud fueron estadísticamente más alto en los caballos que en los ponis y ninguna significativa diferencia estadística se encontró sobre las acrofases y robustez. En conclusión suponemos que las diferencias sobre la cantidad de toda la actividad motora en animales de diferente biotipo, como poni y caballo, podrían ser diferencias físicas.

Palabras clave: actividad motora, *Equus caballus*, masa corporal, ritmo circadiano.

INTRODUCTION

A wide variety of organisms exhibit daily behavioral and physiologic rhythms. These rhythms are mostly generated by an endogenous circadian timing system that is entrained by environmental cues, or zeitgebers. The light/dark cycle is the most potent cue for circadian entrainment in most organisms. Daily rhythms probably evolved as adaptations that allowed organism to prepare for relatively predictable events in their environment (Bradshaw & Holzapfel 2007). Locomotor performance and its daily rhythms have been widely studied and quantified in a variety of organisms. Daily activity patterns have been well described in domestic mammals including rabbits, cow, cats, dogs, sheep (Refinetti 2006, Piccione et al. 2010), goats, (Piccione et al. 2008a, 2008b, 2008c) and horses (Gill 1991, Scheibe et al. 1999, Piccione et al. 2008d). Locomotor performance has been considerate an "ecologically relevant" trait because of its effect on animal's ability to escape from predators or to catch prey (Díaz 2002).

Total locomotor activity, that includes different behaviours such as feeding, drinking, walking, grooming and small movement during sleep, is influenced by different factors such as photoperiod (Bertolucci et al. 2008), different stabling conditions (Piccione et al. 2008e) and feeding schedules (Piccione et al.

2007). It has been previously demonstrated that separate primate species of differing body size differ in locomotor and postural activities and that body size differences within a species can also be correlated with differences in locomotor behavior (Doran 1993). Body size is a major factor in animal ecology and crucial with respect to the mechanical properties of the skeleton for support and locomotion in terrestrial animals (Christiansen 1999). It has been observed that the relationship between locomotor activity and body mass in terrestrial mammals does not follow a single linear trend when the entire range of body mass is considered. Large taxa tend to show different scaling exponents compared to those of small taxa, suggesting that there would be a differential scaling between small and large mammals. This pattern, noted previously for several morphological traits in mammals, has been explained to occur as a result of mechanical constraints over bones due to the differential effect of gravity on small and large-sized forms (Díaz 2002). Few studies investigated the influence of body mass on physiological parameters. In dogs, inverse body temperature values respect to the body size were observed. Large-size breed showed lower rectal temperature than small-size breed. The difference in body size associated with the different breeds was observed to have a strong impact on the absolute level of body temperature regardless of age (Piccione et al. 2009). Breed differences in body temperature have also been reported in cows, as Holstein cows were found to have higher body temperature than Jersey and Brown Swiss cows (Kendall & Webster 2009).

The inter-species variability of motor activity has been investigated in five mammal species that differ in body mass, showing no correlation between body mass and amount of motor activity (Piccione et al. 2010). Considering that, the aim of this study was to test if motor activity is influenced by body mass in animals of the same species that differ in breed, as horses and ponies.

METHODS

In our study, which lasted two days, six clinically healthy ponies (Shetland pony breed, females, 8-10 years old, 180 ± 30 kg body mass) and six healthy horses (Sella Italiana breed, females, 8-10 years old, 530 ± 20 kg body mass) were used. All animals came from the same farm. General animal care was carried out by the same professional staff.

All animals were housed in individual boxes (4.50 x 4.50 m, equipped with big windows) under natural spring photoperiod (sunrise at 6:00 h, sunset at 19:00 h), at an indoor temperature of 18-21 °C, with feeding (hay and oat) provided three times a day, at 7:00, at 12:00 and 18:00 h, and water available ad libitum. Boxes were cleaned in the morning after 7:30 (following the food administration). The visual and acoustic isolation of an animal from the other avoided the social entrainment of daily behavioural rhythms (Davidson & Menaker 2003). Thermal and hygrometric records were collected inside the box for the whole study by means of a data logger (Gemini, UK). Minimal and maximal temperatures during the experimental period were 16 °C and 20 °C, and mean humidity was 60-70 %. The animal was placed in the experimental box or caged 30 days before starting the study to avoid changes in the behaviour and physiology due to the state of fear induced by isolation (Carbonaro et al. 1992).

To record total activity, we equipped the animals with Actiwatch-Mini® (Cambridge Neurotechnology Ltd., UK), actigraphy-based data loggers that record a digitally integrated measure of motor activity. This activity acquisition system is based on miniaturized accelerometer technologies, currently used for human activity monitoring but also tested for activity monitoring in small non-human mammals (Munoz-Delgrado et al. 2004, Mann et al. 2005). Actiwatch-Mini® utilizes a piezo-electric accelerometer that is set up to record the integration of intensity, amount and duration of movement in all directions. The corresponding voltage produced was converted and stored as an activity count in the memory unit of the Actiwatch-Mini®. The maximum sampling frequency was 32 Hz. It is important to stress that due to this improved way of recording activity data there is no need for sensitivity setting as the Actiwatch unit records all movement over 0.05 g. Actigraphs were placed by means of collars that were accepted without any apparent disturbance. Activity was monitored with a sampling interval of 5 min. Actograms, a type of graph commonly used in circadian research to plot activity against time, were drawn using Actiwatch Activity Analysis 5.06 (Cambridge Neurotechnology Ltd., UK). 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 (that is, the time of the daily peak) was competed by cosinor rhythmometry (Nelson et al. 1979) as implement in the Actiwatch Activity Analysis 5.06 program.

Statistical analysis

Data were normally distributed ($P > 0.05$, Kolmogorov-Smirnov's test).Two-way analysis of variance (ANOVA) for repeated measure was used to determine significant differences due to the body mass and time of day ($P < 0.05$ was considered statistically significant). Four parameters were determined: mean level, amplitude, acrophase (time of peak), and robustness (strength of rhythmicity). The amplitude of a rhythm was calculated as half the range of oscillation, which in its turn was computed as the difference between peak and trough. The acrophase of a rhythm was determined by an iterative curve-fitting procedure based on the single cosinor procedure, as described by Nelson et al. (1979). Rhythm robustness was computed as a percentage of the maximal score attained by the chi-square periodogram statistic for ideal data sets of comparable size and 24-h periodicity (Refinetti 2004). Robustness greater than 15 % is above noise level and indicates statistically significant rhythmicity. Unpaired Student t-test was used to evaluate statistical differences between rhythmic parameters of the variable studied in ponies and horses. All treatments, housing and animal care reported above were carried out under the guidelines of the Italian Ministero della Salute for the care and use of animals (D.L. 27/1/1992, n 116) and UE (Directive 86/609/CEE).

RESULTS

Visual inspection of actograms showed that both ponies and horses were mainly diurnal, exhibiting greater activity during the photophase than during the scotophase. During the experimental period total activity was concentrated from 6:30 to 18:30, while during the scotophase there were several activity peaks mostly with lower intensity and shorter than during the photophase, with several cycles of rest/activity (Fig. 1). Table 1 shows mean values and standard deviation of motor activity recorded every hour for 24 during the two days of monitoring in ponies and horses.

The application of two-way ANOVA showed a highly significant effect of time of day and body mass on motor activity in both days of monitoring (Time: day one $F_{24, 240} = 3.791$, $P < 0.0001$; day two $F_{24, 240} = 6.159$, $P < 0.0001$; Size: day one $F_{1, 240} = 11.26$, $P = 0.0073$; day two $F_{1, 240} = 5.265$, $P < 0.0447$). The application of the periodic model and statistical analysis of the cosinor enabled us to define the periodic parameters during the two days of monitoring. Mesor and amplitude were statistically higher in horses than in ponies (Mesor: $t_2 = 9.667$; $P = 0.0105$; Amplitude: $t_2 = 8.201$, $P = 0.0145$ Student's t-test). No statistically significant differences were found about acrophases and robustness. The acrophases were observed at 16:36 and 15:34 in ponies, 13:41 and 14:00 in horses during the days one and two, respectively. The robustness values observed in ponies were 18.66 % and 21.07 %, while in horses were 22.06 % and 30.43 %, during the days one and two, respectively (Fig. 2).

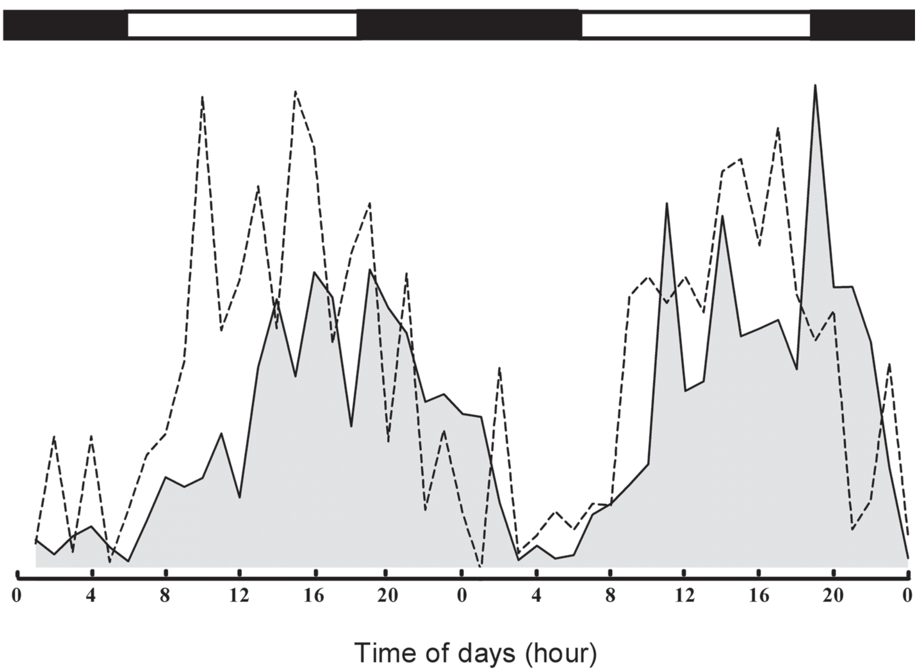

Fig. 1: Pattern (mean) of locomotor activity recorded in ponies (grey area) and horses (dashed line) during two days of monitoring.

Modalidad (medio) de la actividad locomotor registrada en los ponis (área gris) y en los caballos (línea punteada) en los dos días de seguimiento.

TABLE 1

Mean and standard deviation (SD) of motor activity recorded every hour for 24 during the two days of monitoring in ponies and horses.

Valores medios y desviaciones estándares (SD) de la actividad motora grabada cada hora por 24 horas en los dos dias de seguimiento en ponis y caballos.

Time of day (hour)	Ponies				Horses			
	Day 1		Day 2		Day 1		Day 2	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
00:00	26.66	24.19	132.83	160.67	34.00	81.32	0.00	0.00
01:00	13.83	15.14	58.66	72.17	167.33	205.11	253.33	182.38
02:00	29.66	17.27	9.16	14.37	22.50	46.93	21.83	35.74
03:00	38.16	44.57	21.33	47.52	167.83	215.77	44.16	105.27
04:00	20.16	19.06	10.50	25.71	10.83	25.56	74.00	136.10
05:00	8.00	9.38	13.33	25.63	75.66	172.45	50.83	121.60
06:00	43.16	83.62	48.50	113.02	144.66	191.94	83.50	127.45
07:00	80.66	61.68	57.83	58.43	170.33	173.59	81.16	98.12
08:00	72.33	54.13	74.33	76.34	261.00	220.31	342.33	352.41
09:00	80.16	73.55	92.00	74.25	591.33	641.94	367.16	246.87
10:00	118.66	120.31	317.33	348.65	299.66	251.06	334.16	249.76
11:00	63.16	65.55	155.50	224.52	362.83	185.87	366.83	192.67
12:00	175.83	187.26	163.83	185.70	479.66	264.62	322.16	232.12
13:00	234.33	184.47	306.50	222.46	302.00	276.24	498.50	43.50
14:00	168.16	146.92	202.33	211.36	598.16	256.21	513.83	201.76
15:00	258.00	157.06	209.00	296.43	528.50	617.15	406.00	356.48
16:00	236.00	368.71	216.5	186.46	283.33	239.12	553.00	269.16
17:00	124.66	181.29	174.33	177.49	395.16	251.23	344.33	300.94
18:00	260.16	294.57	419.83	167.28	458.33	144.75	286.66	161.60
19:00	227.00	169.50	244.66	159.94	161.33	249.54	324.16	100.34
20:00	205.33	109.31	245.16	96.56	370.66	207.15	51.166	120.02
21:00	146.00	110.74	197.50	357.97	76.00	130.77	87.66	159.43
22:00	152.66	153.02	89.16	202.21	175.50	271.16	259.16	186.17
23:00	135.50	75.33	10.83	22.00	72.50	118.41	44.66	109.41
24:00	132.83	160.67	152.66	209.58	0.00	0.00	107.83	126.65

DISCUSSION

According to previous studies locomotor activity exhibits robust daily rhythmicity in horses (Piccione et al. 2005) and ponies and it was concentrated during the photophase. A statistical difference in the amount of activity was observed between ponies and horses. Horses showed higher amplitude of rhythm of motor activity than ponies. Locomotor behaviour is clearly related to the body size of the species, with increasing size, the amplitude of the movements increase. The results of previous study showed that mammals of different sizes move in a dynamically similar manner. Dynamic similarity is a paradigm that proposes how it is possible for animals of different sizes to move in ways that are easily recognizable as similar (Alexander & Jayes 1983, Alexander 1989). For example, if a horse and a rat trot in a dynamically similar manner, their feet will spend a similar fraction of a stride period on the ground and they will swing their limbs through similar angles. The duty factor (the duration of the time a foot is on the ground during a stride) during fast locomotion increases with body size in ungulates, implying that peak forces during fast locomotion in large animals will be lower multiples of body mass than in smaller forms. In very large mammals, reduced locomotor performance also contributes to maintaining peak stresses in the limbs at comparable levels to those seen in much smaller mammals (Christiansen 1999). Previous study showed that pony foal locomotion characteristics were qualitatively

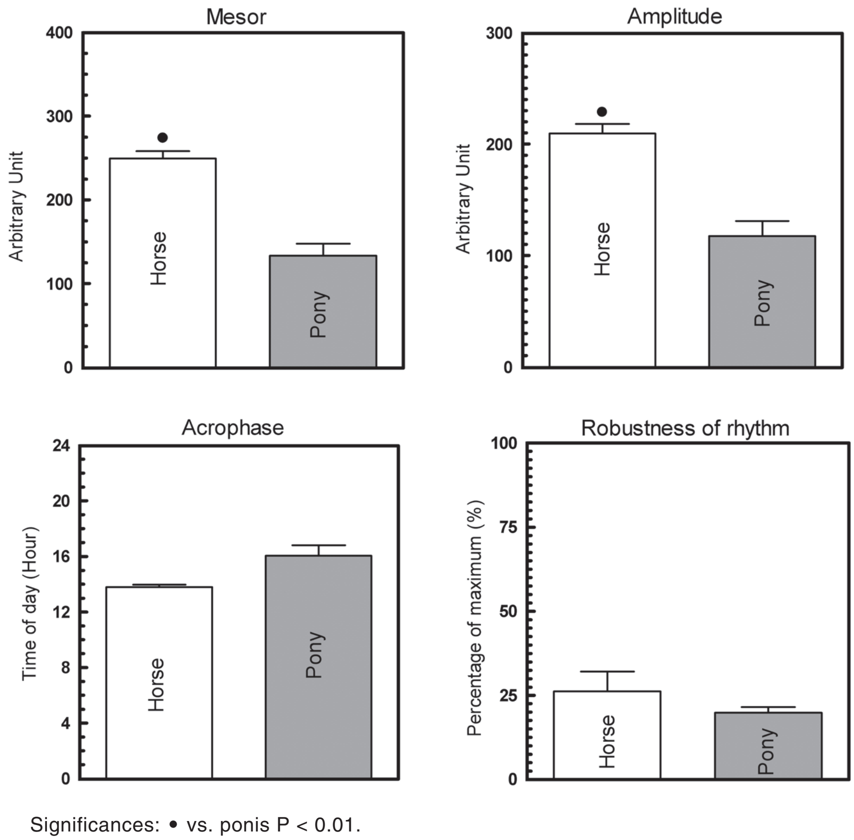

Fig. 2: Mean values (± SD) of Mesor, Amplitude, Acrophase, and Robustness of locomotor activity of ponies (n = 6) and horses (n = 6) • vs. pony P < 0.01.

Valores medios y desviaciones estándares (± DE) de Mesor, Amplitud, Acrofase, y Fuerza de la actividad locomotor de ponis (n = 6) y caballos (n = 6) • vs. ponis P < 0.01.

similar to those of horses. It underlined that ponies made shorter strides than horses, evidenced by a shorter stance and swing duration, although their relative stance durations were similar (Back et al. 1999). Previous studies, whose aim was to evaluate the influence of body size on different variables in mammals, showed that the amplitude of the circadian pattern of body temperature was almost fixed among species of different body weight, while the amplitudes of the circadian pattern of oxygen consumption (VO_2) and heart rate (HR) was fixed despite the huge differences in physical characteristics. Presumably, the amplitudes of these variables are controlled by parameters of physiological and not physical significance (Mortola & Lanthier 2004). Our previous work showed that the rabbits did not exhibit the lower locomotor activity than the other species with a larger size (Piccione et al. 2010) and it confirmed that in different species the amplitude of locomotor activity was not always related to the body size. Instead, the present study showed that in animals of the same specie of different breed, as pony and horse, with the same temperament, the amplitude of motor activity, that appear higher in horses, is correlated with body mass. As we observed, in different species with different mass, the amount of total locomotor activity is not influenced by body mass but feeding behaviour (Piccione et al. 2010). Besides, it has been demonstrated that the daily organization of activity patterns is peculiar to each species, being modulated by (Piccione et al. 2010) social behaviour may influence total locomotor activity.

By submitting the two breeds, which came from the same farm and with the same temperament, to identical environmental conditions, the different mesor and amplitude values of motor activity observed might be attributed to different physical characteristics of breed-related to body mass, which changes quantitatively the amount of activity within 24 hours.

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