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Research

Intrasubject and intersubject variabilities in the daily rhythm of total locomotor activity in horses

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ABSTRACT

The observation of locomotor activity and resting behavior is used to determine time budgets, which may give some indication for the assessment of horses' welfare concerning management and housing. The aim of this study was to investigate total locomotor activity (TLA) daily rhythm in athletic horses (Thoroughbred, Standardbred, and Italian Saddle), with special attention to the intrasubject and intersubject variabilities. Ten subjects for each breed were divided into two subgroups, one housed in box and one housed in paddock. TLA was recorded for 10 consecutive days by mean of an activity data logger, Actiwatch Mini, applied at each horse by mean of a headstall. The obtained data were analyzed using the method of single cosinor. All horses, in all experimental conditions, showed a daily rhythm of TLA. On the obtained circadian parameters (mesor, amplitude, acrophase, and robustness), a multivariate analysis of variance was applied to investigate the effect of breed, housing conditions, and days of monitoring. It showed a statistically significant effect of breed on all circadian parameters; an effect of housing condition on mesor, amplitude, and robustness; and an effect of days of monitoring on acrophase and robustness. The analyses of intrasubject and intersubject variabilities indicate that organization of TLA daily rhythm in Standardbred and Jumper is characterized by greater accuracy of control within and between the individuals of a breed. In contrast, Thoroughbreds showed a high intrasubject and intersubject variability for all parameters studied, except for the intravariability of acrophase, denoting a different response to noxious stimuli that was observed not only among the subjects but also within the subject.

Introduction

Horses are social animals that live in groups in close contact with conspecifics. They spend most of their waking hours moving at a walk, grazing, and eating grass. The domestic environment within which horses are kept and managed can present challenges to instinctive and innate behavior patterns (Minero and Canali, 2009). With domestication, the organization of horse behavior has been demonstrated to remain relatively unchanged suggesting that the constraints imposed on the horse during the last century may be in conflict with their naturally evolved behavior and may lead to welfare problems. The modern domestic horse is shaped by evolution and species-specific behavior and physiology (Christensen et al., 2002; Goodwin, 2002).

Physiological measures routinely used to evaluate welfare include the autonomic response of the animals, such as increase in heart rate and altered levels of various hormones (Korte, 2001). Behavior has the advantage that it can be studied noninvasively and can provide direct insight into the view of the situation from the perspective of an animal. Locomotor behavior has a key role in horses, and it has positive physical and mental effects on them. Their natural attitude for locomotion is an essential consideration in the management of domestic horses (Mills and Clarke, 2002). When horses are kept under natural environmental conditions, the temporal organization of activity and perhaps additional aspects of

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equine physiology rely relatively more on conspecific–social signals than on endogenous circadian regulation and/or external time cues (Murphy et al., 2006). The circadian clock, located in the suprachiasmatic nuclei of hypothalamus, is an evolutionary highly conserved feature of animals that allows organisms to adapt their physiological processes to the time of the day. Circadian rhythms reflect extensive programming of biological activity that meet and exploit the challenges and opportunities offered by the periodic nature of the environment (Pittendrigh, 1993).

Although horse activity and behavior may be greatly influenced, and in some cases driven by external environmental factors including interactions with humans, it is also influenced by endogenous circadian rhythms that exist both centrally and peripherally (Martin et al., 2010). A pervasive feature of endogenous circadian oscillators is the capacity to synchronize rhythms to environmental synchronizers including light, temperature, and food availability (Pittendrigh, 1993).

Synchronization between endogenous daily rhythm and the environment ensures optimum survival of organisms by permitting physiological anticipation of rhythmic environmental changes in light, temperature, humidity, food availability, and predation pressure (Murphy, 2010; Woelfle et al., 2004). Gill (1991) developed the first mythology to study locomotor activity in horses. In later studies, actigraphy-based data logger was used to analyze total activity, including that attributable feeding, drinking, walking, grooming, and small movements during sleep, independent of the animal's position, such as lying or standing. In horses, total locomotor activity (TLA) is influenced by different factors such as photoperiod (Bertolucci et al., 2008; Piccione et al., 2011a), different stabling conditions (Piccione et al., 2008), and feeding schedules (Piccione et al., 2013). Grazing behavior occupies the majority of any horse's time and consists of both locomotor activity and feeding. The time spent grazing depends on the structure and dispersion of patch on which animals prefer to eat, season, age, sex, and herbage availability (Houpt et al., 2001).

It has been previously demonstrated that primate species of different body sizes 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 terrestrials animals (Christiansen, 1999). The relationship between locomotor activity and body mass in terrestrial mammals does not follow a single linear trend. Interspecies variability of locomotor activity has been investigated in five mammal species that differ in body mass, without demonstrating a correlation between body mass and the amount of locomotor activity (Piccione et al., 2010; 2014). Intraspecies variability in the amount of locomotor activity has been in comparisons of horses and ponies. Differences were largely attributable to the amplitude size of movement related to size of animals (Piccione et al., 2011b). Little research has been conducted on intrasubject and intersubject variabilities of daily rhythms, except for activity rhythm studies performed on domestic mouse and Nile grass rat (Refinetti, 2006).

Accordingly, we sought to investigate the TLA daily rhythm in horses of different breeds housed in individual boxes and paddocks, with the intent of focusing on intrasubject and intersubject variabilities.

Materials and methods

Animals and housing

Our study was conducted on 30 clinically healthy horses of three different breeds. Each breed was chosen for a slightly different athletic attitude. The study population included 10 Italian Saddle geldings (Jumpers; 8 ± 2 years, mean body weight = 480 ± 35 kg), 10 Thoroughbred geldings (7 ± 1 years, mean body weight = 460 ± 20 kg), and 10 Standardbred geldings (7 ± 2 years, mean body weight = 440 ± 26 kg). Each group was divided into two subgroups, one was housed in individual boxes (4.00 × 4.00 m^2, equipped with big windows) under the natural photoperiod (sunrise at 06:10, sunset at 18:20 on day 1 and sunrise at 05:55, sunset at 18:30 on day 10) and with natural environmental temperatures. During the study, the box windows were kept open to guarantee good illumination and ventilation. Thermal and hygrometric data were collected inside the box for the entire study period using a data logger (Gemini, UK). These data followed the normal seasonal pattern for the locale (13°C-18°C; 60 ± 10% relative humidity). The horses in the other group were kept individually in a paddock (1.500 m^2) without pasture and with an 80 m^2 pen. Both subgroups were studied during the same time of the year. No statistically significant differences were observed for climatic conditions between boxes and paddock. For all groups, water was available *ad libitum*, and horses were fed three times a day (07:00, 12:00, 19:00) on the basis on nutritional needs of each group. Each group was subjected to investigated management 15 days before the start of the study and was located in the own farm, where they had experience of housing in box and/or paddock. The horses in the box subgroup were put individually into a 20 × 40 m^2 area 1 h/day, randomly, during the morning hours. All treatments, housing, and animal care reported previously were conformed to the standards recommended by the Guide for the care and use of animals (D.L. 27/1/1992, n 116) and UE (Directive 86/609/CEE).

TLA recording

TLA of horses was recorded for 10 consecutive days. Each horse was equipped with an actigraphy-based data logger (Actiwatch Mini, Cambridge Neurotechnology Ltd, UK) that records a digitally integrated measure of motor activity. Actigraphs were placed by means of headstalls that were accepted without any obvious disturbance; in according to previous investigation showing that the behavior parameters of activity were correctly identified by collars (Berger, 1993). This activity acquisition system is based on miniaturized accelerometer technologies; Actiwatch uses a piezoelectric accelerometer that is set up to record the integration of intensity, amount, and duration of movement in all directions. The corresponding voltage produced is converted and stored as an activity count in the memory unit of the Actiwatch. The maximum sampling frequency is 32 Hz. Actigraphs were placed by means of headstall that were accepted without any apparent disturbance. Activity was monitored with a sampling interval of 5 minutes.

Statistical analysis

Using 5-minute data recording intervals (288 data point), cosinor rhythmometry (Nelson et al., 1979) was applied to determine four rhythmic parameters: mesor (mean level), amplitude (half the range of oscillation), acrophase (time of peak), and robustness (strength of rhythmicity).

For each subgroup (horses n = 5), intrasubject and intersubject variabilities in the amount of activity were computed as the standard deviations of the means. The standard deviations of the mean of the five horses across 10 days were used as the measure of intrasubject variability. Likewise, the standard deviations of the means for 10 days across the five subjects were used as the measure of intersubject variability.

A multivariate analysis of variance (ANOVA) for repeated measures was used to determine any statistically significant effect