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Research

Different daily patterns of serum cortisol and locomotor activity rhythm in horses under natural photoperiod

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ABSTRACT

The goal of the present study was to investigate the daily pattern of serum cortisol rhythm in relation to the total locomotor activity in horses housed in an individual box under a 12-hour light-dark cycle. Blood samples were collected at 3-hour intervals over a 48-hour period via an intravenous cannula inserted into the jugular vein in 5 Italian Saddle horses. In addition, the horses were equipped with Actiwatch Mini, actigraphy-based data loggers that record a digitally integrated measure of motor activity. The application of cosinor rhythmometry showed a daily rhythmicity of serum cortisol levels and locomotor activity. Both parameters showed a diurnal acrophase, at the beginning of photophase for cortisol anticipates of about 5 hours with respect to the acrophase of locomotor activity. The data on the distribution of acrophases have implications for the issue of causation. In conclusion, we found no influence of the rhythm of locomotor activity with the rhythm of serum cortisol.

Introduction

Cortisol has been identified as the major free-plasma glucocorticoid in horses. The determination of serum cortisol values has been used to estimate the level of animal stress and welfare in several species, including horses (Broom, 1988; Alexander and Irvine, 1998; Cordero et al., 2012). It is well known that diurnal changes in serum cortisol levels through the day depend on the endogenous rhythmic function of the adrenal cortex. The cortisol rhythm is also depicted as a marker of the circadian temporal organization. Under normal conditions, daily cortisol changes began with an early-morning acrophase, declining throughout the day to reach minimal levels in the evening followed by an abrupt rise in the second half of the night (Weible and Brandenberg, 2002). This function depends on the rate of metabolism and is not influenced by the environmental factors. In pregnant mares, it was observed that changes in diurnal pattern of locomotor activity or different physiological states may cause disturbances in the circadian cortisol level (Flisińska-Bojanowska et al., 1991). The absence of a normal cortisol circadian rhythm is a reliable indicator of chronic stress and poor welfare (Möstl and Palme, 2002). The appearance of reproducible and stable circadian rhythms, with a characteristic phasing with respect to other biological processes and the external environment, is believed to guarantee an optimal functioning of the biological system, with maximum efficiency, performance, and welfare (Weinert and Waterhouse 2007). Johnson and Malinowski (1986) attributed the increase of cortisol during the 2-hour light period supplement exposure with full-spectrum, cool-white fluorescent light in a 10-hour natural daylight to the result of an acute increase in locomotor or feeding activity.

Total locomotor activity (TLA), as changes in the behavioral activity of animals, is widely used as an indicator for the assessment of animal welfare (Müller and Schrader, 2003). The daily rhythm of locomotor activity has been documented in a large number of species of mammals including rabbits, cats, dogs, sheep, goats (Refinetti 2006, Piccione et al. 2010), and horses in relation to different photoperiods, housing conditions, and time of food administration (Bertolucci et al, 2008, Piccione et al. 2008, 2013). Furthermore, in horses, evaluation of the TLA has been also studied in comparison to the daily rhythms of redox state and rectal temperature in trained and untrained subjects (Piccione et al. 2011a,b).

In this regard, considering the importance of cortisol as an index of stress and pathologic conditions and of locomotor activity as an

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indicator for the assessment of animal welfare, the aim of this study was to investigate the daily rhythm of locomotor activity and the possible influence on the rhythm of cortisol in horses housed in individual boxes under natural environmental conditions.

Materials and methods

Five clinically healthy Italian Saddle horses aged between 8 and 10 years, of 500 ± 35 kg body weight, were used in our study carried out in Messina, Italy (latitude: 38°26′ and longitude: 15°59′). All horses were subjected to the same type of management and were housed in individual boxes (4.00 × 4.00 m), equipped with big windows that guarantee good illumination and ventilation. Water was available ad libitum, and hay and oats were provided 3 times a day (7 AM, 12 PM, 8 PM). The animals were subjected to a natural photoperiod (12-hour light and 12-hour dark). Thermal and hygrometric records were carried out inside the box for the whole study by means of a data logger (Gemini Data Loggers Ltd., Chichester, West Sussex, United Kingdom), and they followed the normal seasonal pattern for the place. The day before the start of sampling, the left jugular furrow of each horse was clipped and surgically prepared for placement of indwelling jugular catheters (Terumo, Roma, Italy). The jugular catheter was secured in place with a suture (Vicryl, Ethicon, Somerville, NJ, USA). All data collections were performed by the same technician. General animal care was carried out by professional staff not associated with the research team. Dim red light (<3 lux; 15 W Safelight lamp filter 1A; Kodak Spa) was used for sample collections during the scotophase.

Blood samples were collected every 3 hours over a 48-hour period, starting at 12 AM on day 1 and finishing at 12 AM on day 3, in Vacutainer tubes without anticoagulant (Terumo Corporation, Tokyo, Japan). After standing at room temperature for 20 minutes, the tubes were centrifuged at 3,000 rounds per minute for 10 minutes, and the obtained serum was stored at −25°C until analyzed. Cortisol concentration was measured with IMMULITE 2000 (Siemens Healthcare, Deerfield, IL, USA), which uses a solid-phase competitive enzyme–amplified chemiluminescent immunoassay. All samples were analyzed in duplicate. Samples exhibited parallel displacement to the standard curve. The overall intra-assay coefficient of variation has been calculated to be <5%.

Locomotor activity

TLA pattern was recorded using the Actiwatch Mini (Cambridge Neurotechnology Ltd., Cambridge, United Kingdom) actigraphy-based data logger that records a digitally integrated measure of motor activity. Actigraphs were placed by means of headstalls that were accepted without any obvious disturbance. This activity acquisition system is based on miniaturized accelerometer technologies, and it has been previously used to record locomotor activity in horses (Bertolucci et al., 2008). Activity was monitored with a sampling interval of 1 minute; it was the result of all movements, which include different behaviors such as feeding, drinking, walking, grooming, and small movements during sleep, independent of the animal's position, as lying or standing.

Statistical analysis

All the results were expressed as mean ± standard deviation. Small data sets allowed the use of a specific mathematical and statistical model (De Prins and Hecquet, 1994). Using cosinor rhythmometry (Nelson et al. 1979), 4 rhythmic parameters were determined: mesor (mean level), amplitude (half the range of oscillation), acrophase (time of peak), and robustness (strength of rhythmicity). Rhythm robustness was computed as the quotient of the variance associated with sinusoidal rhythmicity and the total variance of the time series (Refinetti 2004). A paired Student *t* test was applied to compare rhythmic parameters between day 1 and 2 and to compare the 2 parameters studied. P value <0.05 was considered statistically significant. The data were analyzed using the software STATISTICA 8 (Stat Soft Inc.).

Results

Figure 1 represents the pattern of serum cortisol concentration in comparison to the actogram of locomotor activity and the acrophasogram of locomotor activity in a horse.

The application of the method of single cosinor showed a daily rhythmicity of serum cortisol levels and total locomotor activity. No statistical differences between the 2 days of monitoring were observed in rhythmic parameters for both parameters. Both parameters showed a diurnal acrophase, although statistically different (day 1: $t_4 = 5.22$; $P = 0.006$; day 2: $t_4 = 7.14$; $P = 0.002$). It was observed at the beginning of the photophase for cortisol and in the middle of the photophase for locomotor activity (Figure 2). Robustness of rhythm was statistically higher (day 1: $t_4 = 9.68$; $P = 0.0006$; day 2: $t_4 = 6.33$; $P = 0.006$) in cortisol with respect to locomotor activity.

Discussion

Daily rhythmicity is exhibited by many variables simultaneously. The mean levels and amplitude of different rhythms cannot be compared because they refer to distinct physical quantities. Differences in waveform could be compared, but it must be considered in the analysis of phase relationship (Refinetti, 2006).

Our results showed a daily rhythm of cortisol on both days of monitoring, which had the highest concentration during the early period of the photophase as observed in previous studies conducted in horses and dogs (Johnson and Malinowski, 1986; Giannetto et al., 2014). Figure 1 represents the pattern of serum cortisol concentration in comparison to the actogram of locomotor activity and the acrophasogram of locomotor activity in a representative horse. Locomotor activity showed a daily rhythmicity with highest values during the diurnal period, from 6 AM to 6 PM, whereas during the dark period there are several activity peaks mostly with lower intensity and shorter duration than those during the light period, with several cycles of sleep. The presence of a peak may be because of an individual response to noxae, but anyway, the value of the mean daily activity does not change and the acrophasogram confirms that high activity occurs during the central daylight hours.

Our results also indicate that in horses plasma cortisol concentrations and locomotor activity reached their acrophase at different times of the day and exhibited different degree of rhythmicity.

The data on the distribution of acrophases have implications for the issue of causation. Rhythms that phase lead another rhythm cannot be caused by it, unless the phase leads are so great that it actually constitutes a phase lag in the following cycle.

For the issue of internal order in organisms, that is how the various processes in the body relate to each other; another important aspect is the degree of rhythmicity. In our study, serum cortisol showed a percentage of robustness of about 80% as observed in horses housed under different lighting regimes (Giannetto et al., 2012). In our study, locomotor activity showed a low percentage of robustness of rhythms (20%), contrary to that previously observed in this species housed under an artificial photoperiod and indoor ambient temperature, in which it showed a percentage of robustness of about 51.0% (Piccione et al., 2010). The finding that different variables exhibit different degrees of rhythmicity is not surprising as reported in other studies in which