animal

Seasonal variations in daily rhythms of activity in athletic horses

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Circadian rhythms reflect extensive programming of biological activity that meets and exploits the challenges and opportunities offered by the periodic nature of the environment. In the present investigation, we recorded the total activity of athletic horses kept at four different times of the year (vernal equinox, summer solstice, autumn equinox and winter solstice), to evaluate the presence of seasonal variations of daily activity rhythms. Athletic Thoroughbred horses were kept in individual boxes with paddock. Digitally integrated measure of total activity of each mare was continuously recorded by actigraphy-based data loggers. Horse total activities were not evenly distributed over the day, but they were mainly diurnal during the year. Daily activity rhythms showed clear seasonal variations, with the highest daily amount of activity during the vernal equinox and the lowest during the winter solstice. Interestingly, the amount of activity during either photophase or scotophase changed significantly throughout the year. Circadian analysis of horse activities showed that the acrophase, the estimated time at which the peak of the rhythm occurs, did not change during the year, it always occurred in the middle of the photoperiod. Analysing the time structure of long-term and continuously measured activity and feeding could be a useful method to critically evaluate athletic horse management systems in which spontaneous locomotor activity and feeding are severely limited. Circadian rhythms are present in several elements of sensory motor and psychomotor functions and these would be taken into consideration to plan the training schedules and competitions in athletic horses.

Introduction

The circadian clock is an evolutionary highly conserved feature of bacteria, plants and animals; it allows organisms to adapt their physiological processes to the time of day and season in an anticipatory fashion (Daan and Aschoff, 1982; Pittendrigh, 1993). 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). They have been observed at all levels of organization, from the behaviour of mammals to the specific activity of enzymes (Brown and Schibler, 1999; Gachon *et al.*, 2004).

In mammals, circadian rhythms are driven by a timing system comprising a master pacemaker in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus and of peripheral oscillators in most body cells (Yoo *et al.*, 2004; Saper *et al.*, 2005). At the molecular level the circadian clock mechanism consists of interacting positive and negative transcriptional/

translational feedback loops that operate in a cell-autonomous and self-sustained manner (Gachon *et al.*, 2004).

While the core of the circadian molecular clock has been defined in mammals, the knowledge in the regulation of the circannual (annual) clock is very poor (Davis, 1976; Gwinner, 2003). A recent study on sheep suggests an anatomical substrate of circannual clock based in the pituitary gland in which melatonin-receptor-containing cells may operate as key calendar cells, transmitting seasonal temporal information to the endocrine system (Lincoln *et al.*, 2006).

A pervasive feature of endogenous circadian oscillators is the capacity to synchronize (entrain) rhythms to environmental synchronizer (zeitgeber, from German Zeit, 'time'; geben, 'to give') including light, temperature and food availability (Pittendrigh, 1993). The daily light–dark cycle is the major entraining agent for the mammalian circadian system. In temperate zones, the photoperiod markedly changes across seasons and affects mammalian physiology and behaviour (Sumova *et al.*, 2004). For instance, in nocturnal rodents, the duration of locomotor activity reflects the photoperiod as being shorter on long summer than on

short winter days (Puchalski and Lynch, 1991; Elliott and Tamarkin, 1994). The rhythmic production of the pineal hormone melatonin, which is a part of the timekeeping system, is also highly photoperiod-dependent, both in nocturnal and in diurnal animals; the high nocturnal melatonin production is shorter on long summer than on short winter days (Illnerová, 1991). Food availability for only a limited time each day is also a zeitgeber signal for mammalian circadian rhythms (Stephan, 2002; Mendoza, 2007). Interestingly, recent evidence indicates that clock gene expression in the liver and some other peripheral tissues become entrained to periodic meals whereas clock gene expression in the SCN does not appear to be affected by timed feeding (Damiola *et al.*, 2000; Stokkan *et al.*, 2001).

In domestic mammals, many biochemical, physiological and behavioural processes are under circadian control (Piccione *et al.*, 2005; Refinetti, 2006). Daily rhythms of locomotor activity have been documented in a large number of species of mammals such as rodents, rabbits, cats, dogs and sheep (Refinetti, 2006), whereas few investigations have been conducted in horses (Gill, 1991; Berger, 1993; Scheibe *et al.*, 1999). Seasonal variations of locomotor activity in livestock have been poorly investigated. For instance, previous investigation showed the existence of seasonal variations of locomotor activity in the Przewalski horse (*Equus ferus przewalskii*) maintained under semi-reserve conditions (Berger *et al.*, 1999; Arnold *et al.*, 2006).

It is difficult to describe circadian rhythms in variables relevant to exercise, while at the same time provide information on their underlining mechanisms. Not surprisingly therefore, most researches (Atkinson and Reilly, 1996) have concentrated on describing the various rhythms and discussing the impact on real sports competitions, rather than elucidating the root causes of circadian rhythmicity in sport performance (Drust *et al.*, 2005). The present investigation was aimed at testing whether there are seasonal variations of daily activity rhythms in athletic horses and whether they are influenced by feeding schedule. For this purpose, we recorded total activity of athletic Thoroughbred horses maintained under stabled conditions at four different times of the year (vernal equinox, summer solstice, autumn equinox and winter solstice). Knowledge about the daily and seasonal organization of behaviour in athletic horses could have important implications on the planning of their training schedules and competitions.

Material and methods

Five Thoroughbred horses (female, 5 year old, mean body weight 410 ± 40 kg), clinically healthy and not pregnant were used. Body condition score assessments were used to select mares with the same amount of fatty deposits (Martin Rosset, 1990). Animals were housed in Sicily (Italy; latitude 38° 7′ N; longitude 13° 22′ E) under natural photothermoperiodic conditions in individual boxes (4.5 × 4.5 m) with paddock (3.65 × 10 m) devoid of grass. Horses had free access to the paddock. The shell of the boxes and paddocks allowed the

visual isolation of each horse from co-specifics avoiding the social entrainment of circadian behavioural rhythms (Davidson and Menaker, 2003). Boxes and paddocks were located far away from the centre of the farm to limit the influence of normal human activity. Hay and water were available *ad libitum* in the paddock area. Horses were also fed, one time a day at 0800 h, with 1 kg of a mix of cereals (oats and barley).

Horses underwent fitness training 6 days/week with a rest day on Sundays. Training lasted 2 h each day and included walking, trotting, galloping and obstacle jumping. Fitness training and general animal care were carried out by professional staff not associated with the research team. All work presented here comply with current regulations covering animal experimentation in Italy.

In 2006, the total activity of horses (following the definition of Aschoff, 1962) was continuously recorded for 14 days during four different times of the year: vernal equinox from 14th to 27th March 2006 (sunrise at 0526 h to 0546 h, sunset at 1828 h to 1841 h), summer solstice from 15th to 28th June 2006 (sunrise at 0402 h to 0404 h, sunset at 1953 h to 1956 h), autumn equinox from 16th to 29th September 2006 (sunrise at 0512 h to 0523 h, sunset at 1812 h to 1833 h) and winter solstice from 15th to 28th December 2006 (sunrise at 0635 h to 0643 h, sunset at 1707 h to 1713 h). Activities were recorded during the scheduled times of rest between the different fitness training seasons.

Thermal and hygrometric records were carried out inside the box for the whole study by means of a data logger (TH-2500; Gemini Tinytag, Dundee, Scotland). Both temperature and humidity varied during the year (vernal equinox: 17°C, 12°C, 70%; summer solstice: 28°C, 23°C, 68.5%; autumn equinox: 24°C, 21°C, 71%; winter solstice: 15.5°C, 10.5°C, 73%; maximum and minimum temperatures and mean humidity, respectively).

Actiwatch-Mini® (Cambridge Neurotechnology Ltd, Cambridge, UK), actigraphy-based data loggers that record a digitally integrated measure of motor activity were placed on each mare. This activity acquisition system is based on miniaturized accelerometer technologies. It is 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; Piccione *et al.*, 2007). 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 is converted and stored as an activity count in the memory unit of the Actiwatch-Mini®. The maximum sampling frequency is 32 Hz. It is important to realize 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 headstalls that were accepted by horses without any obvious disturbance. Previous investigations showed that the behaviour parameters of activity were correctly identified by collars (Berger, 1993). In the present investigation total activity recorded was the

result of all movements, which include different behaviours such as feeding, drinking, walking, grooming and small movements during sleep, independent of the animal's position, such as lying or standing. 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).

Each data series of total activity was subjected to several analyses. The amount of activity (as sum of all activity counts recorded in each sampling interval of 5 min) either during the whole day or during photophase and scotophase was calculated using Actiwatch Activity Analysis 5.06.

The length of daily period of total activity was calculated by means of χ^2 periodogram analysis (Sokolove and Bushell, 1978). The photic entrainment was evaluated by visual inspection (Pittendrigh and Daan, 1976a and 1976b). The acrophase of daily rhythm (that is, estimated time of day at which peak of rhythm occurs) was computed by cosinor rhythmometry (Nelson et al., 1979) as implemented in the Actiwatch Activity Analysis 5.06.

The Student's t-test and two-way analysis of variance (ANOVA) for repeated measures were used to determine

significant differences. $P < 0.05$ was considered statistically significant. Scheffé's test was applied for post hoc comparison.

Results

Visual inspection of actograms showed that athletic Thoroughbred horse total activities were not evenly distributed over the day, but they were mainly diurnal during the year (Figure 1). These observations are supported by the statistically significant difference in the amount of activity between photophase and scotophase ($t_{279} = 27.03$, $P < 0.0001$, paired Student's t-test).

Two-way ANOVA for repeated measures showed that the daily amount of activity changed during the year ($F_{(3,260)} = 79.4$, $P < 0.0001$; Figure 2), but not among horses ($F_{(4,260)} = 1.2$, $P > 0.3$). The highest daily amount of activity was found during the vernal equinox (4325 ± 95.7; mean $\pm$ s.e., Figure 2), whereas the lowest amount was found during the winter solstice (2609 ± 37.8; $P < 0.01$, Scheffé's post hoc test; Figure 2).

The amount of activity either during photophase or during scotophase significantly changed throughout the year (Photophase: $F_{(3,260)} = 46.3$, $P < 0.0001$; Scotophase: $F_{(3,260)} = 77.9$,
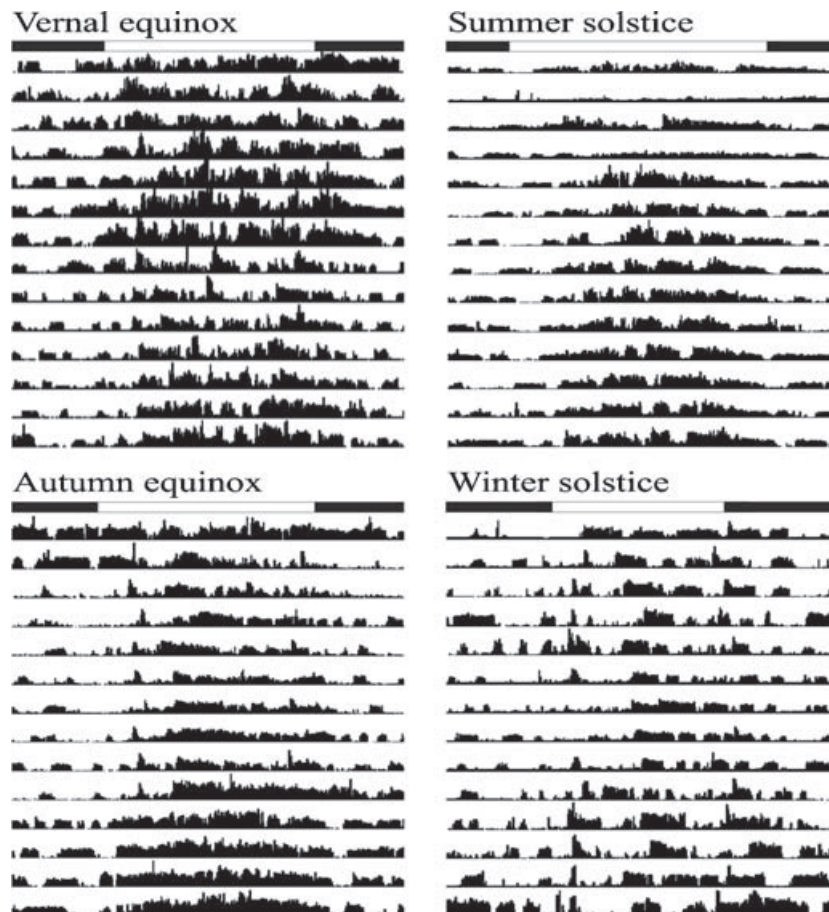
Figure 1 Total activity recorded in an athletic Thoroughbred horse exposed to natural photoperiodic and thermoperiodic conditions during vernal equinox, summer solstice, autumn equinox and winter solstice. The example is representative of the seasonal differences in daily rhythms of activity in the horse. Each horizontal line is a record of 1 day's activity, and consecutive days are mounted one below the other. Total activity recorded during consecutive 5-min periods is indicated by vertical black markings. White and black bars at the top of record indicate photophases and scotophases.
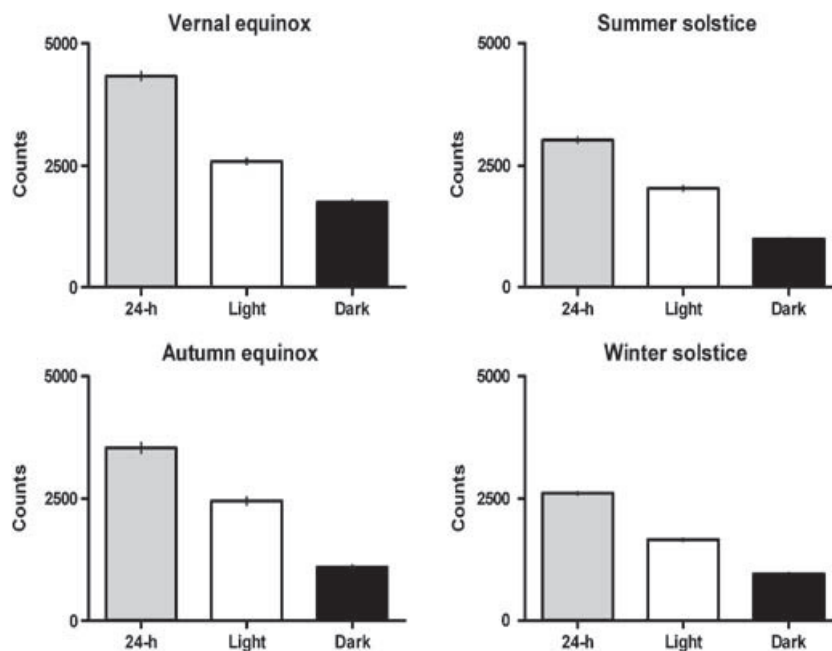
Figure 2 Mean ± s.e. of the daily amount of behavioural activity measured either across the 24-h or in photophase and scotophase at different times of the year.

$P < 0.0001$; two-way ANOVA for repeated measures; Figure 2). *Post hoc* tests showed the highest amount of activity in the photophase during vernal and autumn equinoxes (2583 ± 66.7 and 2447 ± 83.3, respectively; Figure 2) and the lowest during winter solstice (1656 ± 31.5, $P < 0.01$, Scheffé's *post hoc* test; Figure 2). The amount of activity in the scotophase did not change during summer solstice, autumn equinox and winter solstice (991 ± 23.4, 1088 ± 53.7, 953 ± 25.3, respectively; $P > 0.17$, Scheffé's *post hoc* test, Figure 2), whereas during vernal equinox it significantly increased (1742 ± 56.7, $P < 0.01$, Scheffé's *post hoc* test; Figure 2).

During all four times of the year, activity rhythms of the athletic horses showed a period length of 24 h (χ^2 periodogram analysis). Acrophases were comprised between 1210 h and 1510 h (1337 ± 0006 h, mean ± s.e.) in the vernal equinox; 1350 h and 1445 h (1335 ± 0003 h) in the summer solstice; 1225 h and 1500 h (1324 ± 0006 h) in the autumn equinox and 1315 h and 1440 h (1340 ± 0003 h) in the winter solstice (Figure 3). Two-way ANOVA for repeated measures showed that acrophases did not change either among horses ($F_{(4,260)} = 0.08$, $P > 0.9$) or during all times of the year ($F_{(3,260)} = 1.53$, $P > 0.2$).

Discussion

Our results showed the existence of clear daily rhythms of activity during all four times of the year investigated in athletic Thoroughbred horses kept in stabled conditions. Actograms and the distribution of activity over the 24 h underlined the mainly diurnal activity of athletic horses. Our previous investigation in sedentary horses always kept inside their stalls with no physical activity showed robust diurnal activity rhythms (Piccione *et al.*, 2005). The present results
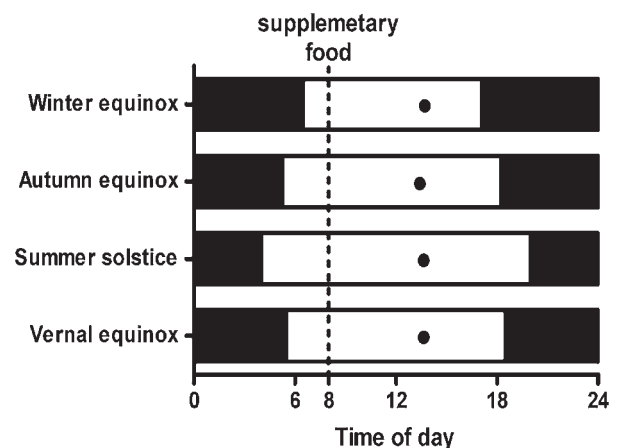
Figure 3 Acrophases of total activity rhythms during vernal equinox, summer solstice, autumn equinox and winter solstice. White and black bars indicate photophases and scotophases. Dotted line indicates hour of supplementary food administration. Note that throughout the year, acrophases always occurred around the middle of photophases.

also confirmed studies in Przewalski horses maintained under semi-reserve, in free-living Camargue and in domestically managed Dutch Warmblood foals in which behavioural activities occurred primarily during daylight time (Boy and Duncan, 1979; Berger *et al.*, 1999; Kurvers *et al.*, 2006).

Athletic Thoroughbred horses had the highest daily amount of activity during the vernal equinox and the lowest during the winter solstice. The highest daily amount of activity during the vernal equinox would depend on the environmental factors such as the increase of photoperiod (Berger *et al.*, 1999; Arnold *et al.*, 2006) and the ambient temperature comprised in the thermoneutral zone

(Morgan, 1998). The lowest amount of horse activity during the winter solstice could be related both to the shortest photoperiods and to the lowest temperatures of the year. In winter, horses face away from wind and seek shelter particularly in raining and wet weather (Duncan, 1985; Boyd and Houpt, 1994). Furthermore, the reduction of total activity could be part of an over-wintering hypometabolic strategy (Arnold et al., 2004 and 2006).

The highest daily amount of activity in the photophase during vernal and autumn equinoxes could be due to the long photoperiod (≈ 13 h). Interestingly, the daily amount of activity in the photophase during the summer solstice, time of the year with the longest photoperiods (≈ 15 h), is lower than during vernal and autumn equinoxes. This result is probably due to the fact that in summer, horses shifted their search for food to the night to avoid disturbance by flying insects and high temperatures (Kaseda, 1983; Mayes and Duncan, 1986; Boyd et al., 1998). Furthermore, in summer, to avoid direct solar radiation, horses often stand in the shade during the warmest parts of the day (Crowell-Davis, 1994).

The significant increase of activity in the scotophase during the vernal equinox could be due to the oestrous behaviour mainly present in the mares between February and June (Evans, 1990). Mares' reproductive performance shows a seasonal trend. It is influenced by photoperiod, through light stimulation that inhibits the synthesis of melatonin and indolic compounds derived from the pineal gland (McDonald, 1989). In the cow, the oestrous behaviour affects the daily activity rhythm, intensifying the frequency of activities at night (Mattner et al., 1974; Hurnik et al., 1975; Esslemont and Bryant, 1976).

In the scotophase, cycles of sleep were interrupted by short activity bouts. As previously observed, these activity bouts had low intensity and ranged from 2 to 15 min (average 7 to 8 min) during which horses lay down in lateral recumbency (Ruckebusch, 1970). Actograms did not give exact information concerning the sleep of the horse or whether it rests in a standing or lying down position (Gill, 1991).

Taken together, our results showed that daily and seasonal variations of total activity rhythms in athletic Thoroughbred horses kept in stabled conditions were similar to those previously found either in sedentary horse or in Przewalski horses (E. f. przewalskii) maintained under semi-reserve conditions (Berger et al., 1999; Piccione et al., 2005; Arnold et al., 2006).

Circadian analysis of horse activities showed that the acrophase, the estimated time at which the peak of the rhythm occurs, did not change during the year. They always occurred in the middle of photoperiod. This result indicates no relationship between the acrophase of activity rhythm and the time of sunrise, which significantly changes during the year. The peak of the rhythm would be determined by the supplementary food always given to the horses one time a day at 0800 h throughout the year. Time of feeding is a potent zeitgeber for peripheral oscillators with only weak synchronizing influence on the SCN clockwork (Damiola

et al., 2000). Under periodic food-availability conditions, this prediction is manifested in anticipatory bouts of loco-motor activity and body temperature (Mistlberger, 1994; Challet et al., 1997; Stephan, 2002). This process seems to be driven by a food-entrainable oscillator independent of the main, light-entrainable clock located in the SCN (Stephan, 2002; Mendoza, 2007). Our previous investiga-tion in a domestic species, the sheep, demonstrated the strong impact of the periodic food availability on the daily rhythms of total activity (Piccione et al., 2007). However, the present experimental protocol cannot permit to verify whether activity rhythms can be differentially entrained according to distinct, sometimes conflicting, temporal (time of sunrise and feeding) cues. Further researches in horses will point to test the impact of timed restricted feeding on the behavioural circadian organization.

In horses, the time spent grazing depends on the struc-ture and dispersion of patches on which animals prefer to eat. This behaviour occupies the majority of its time and it consists of locomotor activity as well as feeding. In horses, the amount of time spent grazing varies with season, age, sex and herbage availability (Houpt et al., 2001). The daily pattern of grazing is altered when it is necessary to adjust for climatic conditions, and to maintain grazing time and thus food intake. Changes in the daily pattern of grazing, and time spent grazing, are the behavioural response of the animal to the physiological effects of climate and to the amount of feed available (Arnold, 1985).

Our data can be seen as baseline data for athletic Thoroughbred horse management and, possibly, for other breeds managed in a similar manner. Analysing the time structure of long-term and continuously measured activity and feeding could be a useful method to critically evaluate athletic horse management systems in which spontaneous locomotor activity and feeding are severely limited. Circa-dian rhythms are present in several elements of sensory motor and psychomotor functions and these would be taken into consideration to plan the training schedules and competitions in athletic horses.

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