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## The Circadian Rhythm of Body Temperature of the Horse

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G. Piccione<sup>1</sup>, G. Caola<sup>1</sup> and R. Refinetti<sup>2</sup>

<sup>1</sup>Dipartimento di Morfologia, Biochimica, Fisiologia e Produzioni Animali, Facoltà di Medicina Veterinaria, Università degli Studi di Messina, I-98100 Messina, Italy;

<sup>2</sup>Circadian Rhythm Laboratory, University of South Carolina, Walterboro, SC 29488, USA

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### Abstract

Rectal temperature of 10 female adult horses was recorded every 2 h for 10 consecutive days under a natural winter photoperiod (9 h of light and 15 h of darkness per day). A robust daily rhythm of body temperature was observed in all animals. The rhythm had a mean level of 38.3°C and a range of excursion of 1.0°C. Temperature started its daily ascent at dawn each day and reached a maximum 14 hours later. Body temperature of 5 of the horses was studied for 10 more days under constant illumination. The rhythm persisted under this condition, although with a slightly longer period of 24.2 h, which confirms the endogenous nature of the rhythm. Despite the fact that the body size of the horse is several orders of magnitude greater than that of rodents, the various parameters of the body temperature rhythm of the horse are similar to those of several species of rodents previously studied.

**Keywords:** Circadian rhythm, body temperature, rhythm robustness, rhythm amplitude, *Equus caballus*.

### Introduction

Circadian rhythmicity is a ubiquitous property of the physiology and behavior of all known organisms (Turek & Van Reeth, 1996). Among the many variables that exhibit circadian rhythmicity, body temperature has received considerable attention (Refinetti & Menaker, 1992). Two important parameters of the body temperature rhythm are its amplitude and robustness. The amplitude refers to the full (or half) range of excursion of body temperature within a cycle. Robustness, which is independent of amplitude, refers to the strength or regularity of the rhythm.

The circadian rhythm of body temperature has been studied in a variety of mammalian organisms — including farm animals —, but most investigations have been conducted in small animals with body size under 10 kg (Refinetti & Menaker, 1992). Greater stability of core temperature is to be expected in large mammals because of their small surface/volume ratio, which contributes to thermal inertia (Aschoff, 1982). Although no relationship was found between body size and the amplitude of the body temperature rhythm in 10 species of mammals ranging in body mass from 30 to 600 g (Refinetti, 1999a), the robustness of the rhythm in the same 10 species was found to be greater in larger animals (Refinetti, 1998). These findings seem to suggest that the greater thermal inertia provided by greater body mass does not reduce the amplitude of the body temperature rhythm but instead shields the endogenously-generated oscillation from disturbances created by the environment.

Although measurements of body temperature rhythm have occasionally been obtained in large mammals such as horse (Hamra et al., 1993) and cattle (Araki et al., 1987), controlled experiments with detailed chronobiological data analysis are lacking. In the present study, we measured and analyzed the body temperature rhythm of horses maintained under a light-dark cycle as well as under constant illumination. At an average body mass of 700 kg, horses are three orders of magnitude larger than the largest mammal used in the previous comparative study (Refinetti, 1998). Consequently, they constitute excellent experimental subjects for extension of the study of the relationship between body size and the amplitude and robustness of the body temperature rhythm in mammals.

## Materials and Methods

Ten female Thoroughbred horses (*Equus caballus*), 8 to 10 years old, were used. The animals were maintained in individual stalls with water available at all times. As per standard horse husbandry, food was not available ad libitum. However, hay and oats were provided four times a day (at 07:00, 12:00, 17:00, and 22:00 h) to avoid the presentation of a conspicuous 24 h temporal cue. Although the multiple feedings did provide a temporal structure, the period of the feeding cycle (approximately 5 hours) was outside the range of entrainment of the mammalian circadian system. Ambient temperature was  $13 \pm 1^\circ\text{C}$ .

The ten animals were studied under a natural winter photoperiod (sunrise at 08:00, sunset at 17:00 h) for 10 consecutive days. Five of the animals were studied for 10 more days under constant illumination (350 lux). Measurements of body temperature were conducted every two hours with a digital thermometer whose probe was inserted 15 cm into the rectum.

For each individual animal in each 10-day segment, the mean level of body temperature was calculated as the arithmetic mean of all 120 data points. Since no outliers were observed in the records (e.g., Fig. 1), the range of excursion of the rhythm was computed as the difference between the highest and the lowest temperatures in the data set.

For computation of the acrophase (time of the daily peak of the temperature rhythm), the 10 days of data were first averaged for each animal. Cosine waves were

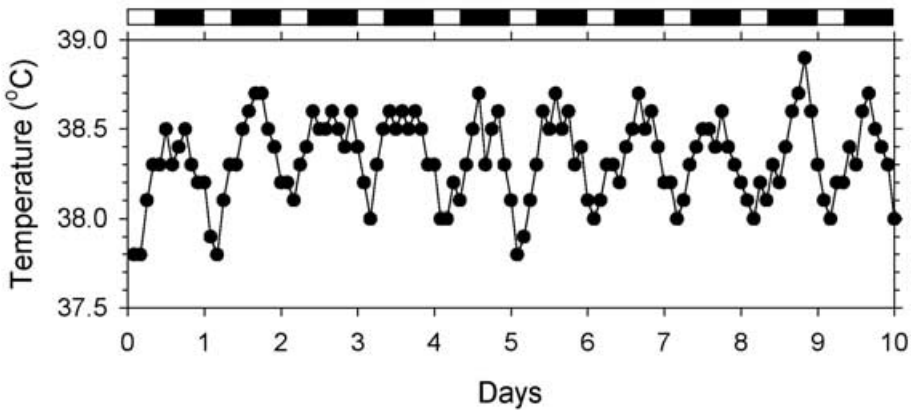


Figure 1. Records of body temperature of a representative horse taken at 2h intervals over 10 consecutive days. The white and black bars at the top of the figure indicate the duration of the light and dark phases of the light-dark cycle, respectively.

then fitted to the averaged 24h rhythm. The time corresponding to the peak of the best-fitting cosine wave was taken as the acrophase of the rhythm.

Determination of circadian period and quantitative analysis of rhythm robustness were conducted by two independent procedures: the chi square periodogram procedure (Sokolove & Bushell, 1978) and the Lomb-Scargle periodogram procedure (Ruf, 1999). The robustness of a rhythm is expressed as the  $Q_p$  statistic and the PN statistic, respectively. These statistics reflect the strength or regularity of a rhythm independently of its amplitude. Circadian period is the period associated with the highest statistically-significant  $Q_p$  or PN in the periodograms.

## Results

The records of body temperature of a representative horse maintained under a natural photoperiod are shown in Figure 1. Clear daily rhythmicity is evident. Temperature oscillates between 37.8°C and 38.9°C with a mean of 38.3°C. The trough of the temperature rhythm consistently occurs at the beginning of the light phase.

Mean body temperature ( $\pm$ SE) for the 10 animals was  $38.32 \pm 0.01^\circ\text{C}$ . The mean range of excursion was  $1.02 \pm 0.03^\circ\text{C}$ . As expected in view of the presence of a 24h light-dark cycle, circadian period was found to be 24.0h by both the chi-square periodogram and the Lomb-Scargle periodogram procedures. Rhythm robustness was  $78 \pm 2$  units according to the chi-square periodogram procedure and  $33 \pm 1$  units according to the Lomb-Scargle periodogram procedure. Interpretation of these values is facilitated by knowledge that maximal robustness corresponds to 120 units in the chi-square periodogram and to 60 units in the Lomb-Scargle periodogram. Thus, the robustness of the body temperature rhythm of the horse as determined in individual animals in this study is 55 to 65% of maximal robustness ('perfect rhythmicity').

To facilitate visual inspection of the synchronization between the body temperature rhythm and the light-dark cycle, Figure 2 shows the grand means of body temperature calculated by first averaging the body temperature readings at each time point over the 10 days for each animal and then averaging the data for the 10 animals. Due to the averaging of values from 10 different animals, the composite range of excursion of body temperature is smaller ( $0.49^{\circ}\text{C}$ ) than that calculated by averaging the ranges of excursion of the individual animals ( $1.02^{\circ}\text{C}$ ). The composite temporal course of body temperature, however, reflects well the temporal course observed in the records of the individual animals. The trough (nadir) of the rhythm is observed at the beginning of the light phase, whereas the acrophase is very close to the mean of the acrophases for the individual animals ( $22:00\text{h}$ ). It can also be seen that the feeding episodes (arrows) had no detectable disruptive effect on body temperature.

The body temperature rhythm persisted in all 5 animals maintained in constant light for 10 additional days, as exemplified by the records shown in Figure 3. Although the records for this animal lack a clear trough between days 4 and 5, they are otherwise very similar to those shown in Figure 1. Neither the main level ( $38.30 \pm 0.01^{\circ}\text{C}$ ) nor the robustness ( $Q_p = 74 \pm 4$ ,  $PN = 31 \pm 2$ ) of the rhythm of the animals kept in constant light differed from those of the animals kept under the light-dark cycle

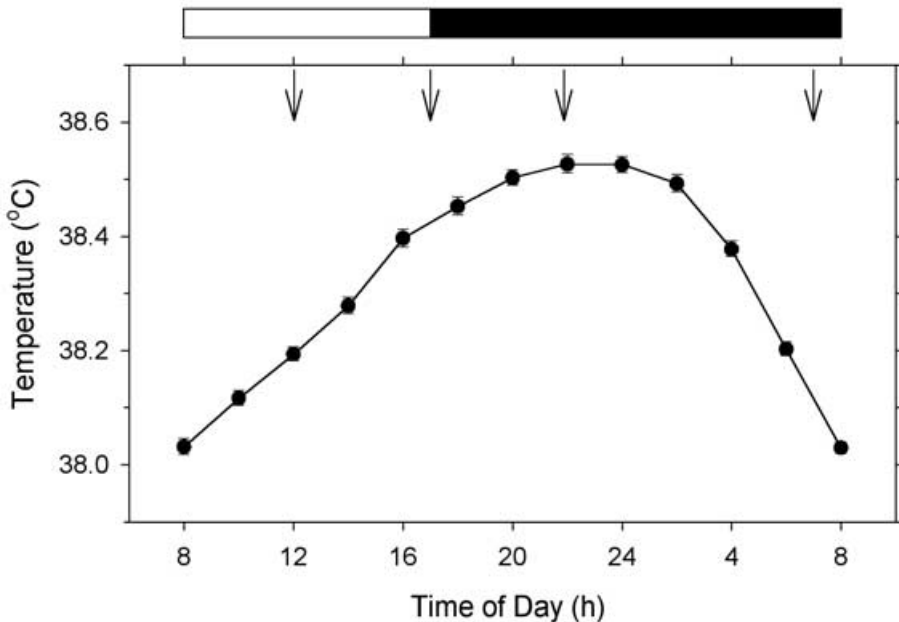


Figure 2. Mean ( $\pm\text{SE}$ ) body temperature of 10 horses, each averaged over 10 days, as a function of time of day. The white and black bars at the top of the figure indicate the duration of the light and dark phases of the light-dark cycle, respectively. The arrows indicate feeding times.

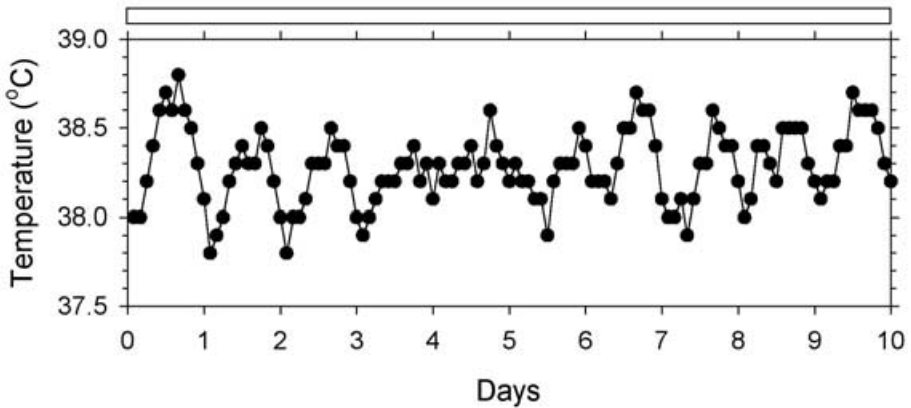


Figure 3. Records of body temperature of a representative horse taken at 2h intervals over 10 consecutive days. The white bar at the top of the figure indicates that the lights were continuously on.

(t tests,  $p > 0.10$ ). The range of excursion of body temperature ( $0.84 \pm 0.05^\circ\text{C}$ ) was significantly smaller than that of animals kept under the light-dark cycle ( $p < 0.01$ ), whereas the period of the rhythm ( $24.2 \pm 0.05\text{ h}$ ) was slightly but significantly longer ( $p < 0.04$ ).

## Discussion

The results indicate the presence of a clear daily rhythm of body temperature in horses maintained under a natural photoperiod. The mean core temperature of  $38.3^\circ\text{C}$  is within the  $36\text{--}39^\circ\text{C}$  range of core temperatures in placental mammals (Cossins & Bowler, 1987). The range of excursion of  $1.0^\circ\text{C}$  is narrower than that of some smaller mammals but equivalent to that of others. From Table 1 in Refinetti and Menaker's (1992) literature review, ranges of excursion of the body temperature rhythm can be calculated as  $1.6^\circ\text{C}$  in rats (mean of 16 studies),  $0.8^\circ\text{C}$  in cats (3 studies),  $2.2^\circ\text{C}$  in dogs (1 study) and  $1.0^\circ\text{C}$  in humans (16 studies).

The robustness of the body temperature rhythm of the horse (65% of maximal robustness when calculated by the chi-square periodogram procedure) is greater than that of some smaller mammals but not that of others. In Refinetti's (1998) study, which used the chi-square periodogram procedure, robustness was approximately 50% in small rodents such as the Siberian hamster (*Phodopus sungorus*, 35 g) and Mongolian gerbil (*Meriones unguiculatus*, 60 g) and approximately 70% in larger rodents such as the Sprague Dawley rat (*Rattus norvegicus*, 500 g) and the Richardson's ground squirrel (*Spermophilus richardsonii*, 600 g). The results in the horse seem to indicate that the increase in the robustness of the body temperature rhythm as a function of body mass found in small mammals (Refinetti, 1998) does not extend to large mammals.

Body temperature of the horse under a light-dark cycle was found to start its daily ascent at the time of lights on (Fig. 2), which is in agreement with the findings in 4 species of small diurnal mammals (Refinetti, 1999b). The acrophase of the rhythm occurred 14 h later, during the dark phase of the light-dark cycle. The nocturnal occurrence of the acrophase may be due to the use of a winter photoperiod (short light phase). Assuming the same time of initiation of the ascent (time of lights on), and the same duration of ascent (14 h), the acrophase would occur slightly before lights off under a summer photoperiod with 15 h of light and 9 h of darkness per day.

The endogenous nature of the body temperature rhythm of the horse was confirmed by the persistence of the rhythm under constant light. The fact that the period of the rhythm was not exactly 24.0 h excludes the possibility of entrainment by temporal cues other than the light-dark cycle. The observed circadian period of 24.2 h is well within the range of circadian periods observed in other mammalian species (Refinetti & Menaker, 1992), although it is unlikely to reflect the exact free-running period of the circadian pacemaker of the horse because exposure to constant light (as opposed to constant darkness) shortens the circadian period of diurnal mammals (Aschoff, 1979). The exact period can only be determined by direct measurements under constant darkness, which could not be conducted in the present study.

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