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Short communication

The effect of photic entrainment and restricted feeding on food anticipatory activity in *Ovis aries*

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ABSTRACT

The aim of this study was to investigate the influence of light/dark (L/D) cycle, associated with restricted feeding, on total daily locomotor activity in sheep. Eight clinically healthy Comisana ewes were housed individually in a soundproof box and subjected to three different light and feeding periods. During period 1 (light on 8:00; light off 20:00) and during period 2 (light on 20:00; light off 8:00) hay and water were available *ad libitum*. During period 3 the animals were subjected to constant light and restricted feeding. Total locomotor activity was recorded by means of an Actiwatch-Mini actigraphy-based data loggers. The application of one-way for repeated measures ANOVA showed a significant effect of 24 h ($P < 0.0001$) on locomotor activity. The highest daily amount of locomotor activity was observed during the photophase than the scotophase ($P < 0.001$). During all three periods locomotor activity exhibited robust daily rhythmicity with diurnal acrophases. During period 3 locomotor activity started before food supplement and it was concentrated during food availability. In conclusion, we can claim that the daily rhythm of locomotor activity in sheep is entrained by the light/dark cycle in couple with food availability.

1. Introduction

The circadian timing system generates daily rhythms of behaviour that permit organisms to anticipate predictable events in their environment, thus increasing the probability of locating essential resources, avoiding predators, and ultimately increasing the likelihood of survival (Holmes and Mistlberger, 2000). Suprachiasmatic neurons drive the central circadian clock which is reset by lighting cues captured and integrated by the melanopsin cells of the retina. The mammalian circadian system consists of at least two major oscillator systems, one entrainable by light, called the central clock and located in the suprachiasmatic nucleus (SNC), and another by food, which is anatomically and functionally distinct but of unknown location, called food entrained oscillator (FEO) (Pardini and Kaeffer, 2006). These clock mechanisms are formed by transcription–translation feedback loops of several interacting genes better known as clock genes (Escobar et al., 2009). Clock gene expression in the liver and other peripheral tissues is entrained by periodic meals (Stephan, 2002). Food access during restricted time of day has profound effects on the behaviour and physiology of animals, which are able to anticipate feeding time (Mistlberger, 1994; Stephan, 2002). In the hour preceding food administration increases in various behavioural and physiological parameters, including body temperature, plasma corticosteron and activity level (food anticipatory activity—FAA) were observed. The capacity to estimate time also gives animals the advantage of anticipating the coming feeding opportunity by approaching the right location and

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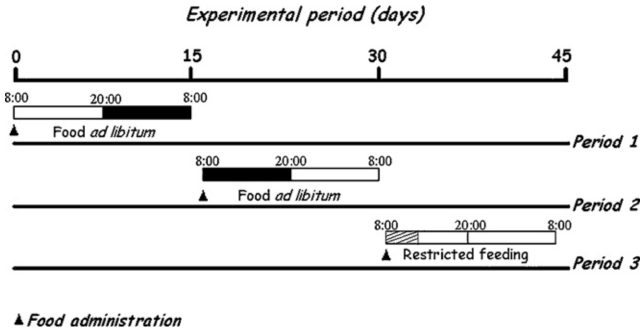

Fig. 1. Summary of experimental conditions. White a black bars indicate photophase and scotophase, the hatched area indicate the restricted feeding period.

to prepare physiological and digestive functions for the expected meal. Circadian clock defines the daily rhythms of locomotor activity and associates physiological regulatory pathways, such as feeding and metabolism (Pardini and Kaeffer, 2006). In rats locomotor activity preceding food access could rely on a biological clock (Bolles and de Lorge, 1962), it was only observed when feeding schedules had a period close to 24 h (Escobar et al., 2009). Among farm animals, the effect of restricted feeding on the behavioural circadian organization has been investigated in sheep (Piccione et al., 2007) and goats (Giannetto et al., 2010). Daily organization of activity patterns is peculiar to each species, being modulated by both external and internal factors (Boy and Duncan, 1979; Nielsen, 1984; Pépin et al., 1991). Particularly, environmental factors may play crucial role in timing the physiology of sheep (Piccione et al., 2009). Our objective was to investigate the influence of photoperiod, associated with restricted feeding, on total daily locomotor activity in sheep.

2. Materials and methods

Eight clinically healthy and not pregnant female sheep (Comisana breed, 2 years old, mean body weight 44 ± 1 kg) were used. Animals were housed individually in a soundproof box of 12 m^2 equipped with an airflow system that maintains the environmental values of the temperature and humidity, 18–21 °C and 50–60% respectively. During the experimental trial all animals were subjected to three different light and feeding periods lasted 15 days each (Fig. 1). During period 1 the light was turn on from 8:00 to 20:00, hay and water were available *ad libitum*; during period 2 the light was turn on from 20:00 to 8:00, hay and water were available *ad libitum*; during period 3 the light was stayed on throughout the monitoring period and the animals were subjected to food restriction. Sheep had access to food only from 8:00 to 13:00, the amount food administered (1.6 kg/day of hay and 0.3 kg/day of commercial concentrates) was calculated on the basis of physiological and productive categories and feeding typology (Avondo, 2005).

Full-spectrum cool fluorescent tubes (FH HE/860 Lumilux T5, Osram GmbH) placed in the middle of the box at 2.5 m height from the floor were used as light source. To record 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. The data logger was placed by means of a neck collar that was accepted by the animals without any apparent disturbance. 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. 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 statistical analysis was applied on the mean values of all 60 data points within each 1 h.

One-way repeated measures analysis of variance (ANOVA) was applied to assess the effect of time of the day on total locomotor activity. Two-way analysis of variance (ANOVA) was used to determine significant differences on the amount of activity during the photophase and scotophase and statistical differences due to the different experimental conditions ($P < 0.05$ was considered statistically significant). We applied a trigonometric statistical model to each time series (25 data points) to describe the periodic phenomenon analytically, by characterizing the main rhythmic parameters according to the single cosinor procedure (Nelson et al., 1979). Four rhythmic 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 on 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. 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. One-way ANOVA was applied on the rhythmic parameters to assess differences due to experimental conditions.

3. Results

The application of one-way repeated measures ANOVA showed a high significant effect of 24 h ($P < 0.0001$) on locomotor activity in all 15 days of monitoring during the 3 experimental periods.

Visual inspection of actograms showed that sheep total activities were not evenly distributed over the day, but they were mainly diurnal. This observation was supported by the results obtained by two-way ANOVA that showed the highest daily amount of activity during the photophase than during the scotophase in all 3 experimental periods ($P < 0.001$). No statistical differences among the 3 experimental periods on the amount of activity during the photophase and the amount of activity during the scotophase were observed ($P < 0.17$) (Fig. 2).

The application of the periodic model and statistical analysis of the cosinor procedure throughout the studied