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ORIGINAL ARTICLE

Locomotor activity and serum tryptophan and serotonin in goats: daily rhythm

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Summary
The goal of the present study was to investigate the daily amount of motor activity and the daily rhythm of tryptophan and serotonin in goats housed in individual boxes under a 12/12 light/dark cycle. We equipped six Girgentana breed goats with Actiwatch-Mini® (Cambridge Neurotechnology Ltd., UK), actigraphy-based data loggers that record a digitally integrated measure of motor activity. Also blood samples were collected at four-hour intervals over a 48 h period via an intravenous cannula inserted into the jugular vein. The concentration of tryptophan (TRP) and serotonin (5-HT) were assessed by the high-performance liquid chromatography (HPLC) method. Our results outline a diurnal pattern of motor activity and levels of tryptophan in the serum, and a nocturnal pattern of levels of serotonin in the serum, which underline the impact of endogenous serotoninergic activity on the behavioural aspects of the circadian response to light.

Key words: motor activity – tryptophan – serotonin – daily rhythm – *Capra hircus*

INTRODUCTION

In mammals many behavioural and physiological processes display 24-hour rhythms that are controlled by the biological clock located in the hypothalamic suprachiasmatic nuclei (SCN) (Reppert and Weaver 2001, Berger 2004).The light:dark (L/D) cycle is the most potent synchroniser (zeitgeber) for circadian entrainment in most organisms, even though in several species, the regulation of circadian timing by behavioural (so-called non-photic) stimuli is well documented. In this case the major input to the SCN is provided by the serotoninergic projections arising from the mesencephalic raphe nuclei (RN). Both photic and non-photic cues are processed by the intergeniculate leaflets (IGL) of the lateral geniculate complex, which are considered as an integrative relay in the circadian system (Malek et al. 2004). The specific destruction of these structures block the synchronization of many circadian physiological processes, such as the phase shifting effect of 8-hydroxy(dipropylamino)-tetraline (a selective serotonin agonist) on the locomotor activity (Schuhler

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et al. 1999). Serotonin (5-HT) is an important neurotransmitter and plays important roles in many physiological functions, including the operation of the mammalian circadian clock (Lovenberg et al. 1967, Prosser 2003). 5-HT is synthesized from the amino acid tryptophan hydroxylase (TPH) and aromatic L-amino acid decarboxylase (Jéquier et al. 1969, Borjigin et al. 1995). 5-HT is a metabolic precursor of melatonin in the pineal gland and is believed to be involved in the control of sleep and in clock resetting (Ganguly et al. 2002). The process is regulated by light signals following a circadian rhythm with maximum levels during darkness and minimum during the light period (Sánchez et al. 2004). It was observed that oral administration of L-tryptophan in different species of animals increases the serum levels of melatonin (Hajak et al. 1991, Huerther et al. 1992, Herichova et al. 1998, Cubero et al. 2006).

Daily rhythms of locomotor activity have been documented in a large number of species of mammals such as rodents, rabbits, cats, dogs, sheep and horses (Gill 1991, Scheibe et al. 1999, Refinetti 2006, Piccione et al. 2007a, b; Bartoszewicz and Barbacka-Surowiak 2007, Piccione et al. 2008).

The present investigation was aimed at testing the daily rhythm of total motor activity and the daily rhythm of TRP and 5-HT in the plasma of goats housed in individual boxes under a 12/12 light/dark cycle.

MATERIALS AND METHODS

Animals

Six females Girgentana breed goats, 20 months old, mean body weight 44.0 ± 1.0 kg, clinically healthy, not pregnant and not lactating, were used. Animals were housed individually in a soundproof light-tight box of 12 m^2 equipped with a darkened opening and an airflow system. The visual and acoustic isolation of each animals from the other one avoided the social entrainment of circadian behavioural rhythms (Davidson and Menaker 2003). The animals were put in the experimental box 30 days before the start of the study to avoid changes in the behaviour and physiology of the animals due to the state of fear induced by isolation (Carbonaro et al. 1992). Thermal and hygrometric records were carried out inside the box for the whole study by means of a data logger (Gemini, UK). The temperature during the experimental period was 15.5 °C minimum; 18.5 °C maximum; and the mean humidity was 55–60%. Animals were kept under artificial 12:12 L/D cycle (L:600 lux at the level of the head of animals; D: 0 lux). During the light phase, full-spectrum cool fluorescent tubes (98 lux) (Osram, Germany) placed in the middle of the box at 2 m height from the floor were used as the light source. As standard farming practice, the animals had free access to water and to good-quality alfalfa hay (90.0 % DM, 15.8 CP % DM, 50.4 NDF % DM, 31.6 ADF % DM, 5.8 lignin % DM, 2.2 EE % DM). Concentrate (oats 23%, corn 36%, barley 38%, and mineral and vitamin supplement 3%) was provided once daily (200 g per animal per day).

Procedures

To record the total activity of the goats, to include different behaviours such as feeding, drinking, walking, grooming and small movements during sleep, we equipped the animals with Actiwatch-Mini® (Cambridge Neurotechnology Ltd., UK), actigraphy-based data loggers that record a digitally integrated measure of motor activity. 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 the amount duration and intensity 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 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. Actigraphs were attached using collars that were accepted without any apparent disturbance. Activity was monitored with a sampling interval of 5 minutes. 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).

Also blood samples were collected at 4-hour intervals over a 48-hour period (starting at 08:00 on day 1 and finishing at 04:00 on day 2) via a jugular intravenous catheter into Vacutainer tubes (Terumo Corporation, Japan) without anticoagulant. Blood samples were immediately centrifuged for 10 min at 3000 rpm with a standardized procedures and, after refrigeration, storage at + 4 °C (maximum of 24 h), the individual serum samples were deproteinized with 5-sulfosalicylic acid (5-SSA), centrifuged for 10 min at 3000 rpm and immediately processed. In the filtered supernatant (20 μm filter), the concentration of tryptophan (TRP) and serotonin (5-HT) was assessed by the high-performance liquid

chromatography (HPLC) method. All housing and care conformed to the standards recommended by the Guide for the Care and Use of Laboratory Animals, and Directive 86/609 CEE.

Statistical analysis

All the results were expressed as mean ± SD. Data were normally distributed ($p<0.05$, Kolmogorov-Smirnov test). One-way repeated measures of analysis of variance (ANOVA) were used to determine the significant effect of time on tryptophan and serotonin (p values < 0.05 were considered statistically significant) and the t-test was used to compare motor activity during the photophase and scotophase. Data were analyzed using the software STATISTICA 7 (StatSoft Inc., USA). The total daily amount of activity, amount of activity during the photophase and the scotophase were calculated using the Actiwatch Activity Analysis 5.06 (Cambridge Neurotechnology Ltd, UK). In addition, we applied a trigonometric statistical model to the average values of each time series, so as to describe the periodic phenomenon analytically, by individuating the main rhythmic parameters according to the single cosinor procedure (Nelson et al. 1979).

Table 1. **Means, SEM and statistical analyses (ANOVA and Cosinor) of tryptophan and serotonin in goats during the 48 h of experimentation**

		Mean	SD	$F_{(5,25)}$	P	Robustness	Φ	A
Tryptophan	Day 1	22.74	1.07	3.414	0.0173	87.80	12:39	2.61
	Day 2	22.88	1.07	4.548	0.0044	86.60	11:17	2.85
Serotonin	Day 1	3.04	0.27	6.917	0.0004	79.50	20:31	0.49
	Day 2	3.03	0.22	3.923	0.0092	90.7	21:10	0.37

Four rhythmic parameters were determined: mean level, amplitude, acrophase (time of peak), and robustness (strength of rhythmicity). For each parameter, the mean level of each rhythm was computed as the arithmetic mean of all values in the data set (12 data points); the amplitude of a rhythm was calculated as half the range of oscillation, which in its turn was computed as the difference between peak and trough. 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 70% is above noise level and indicates statistically significant rhythmicity.

RESULTS

The total daily amount of activity was 652.5±39.34 activity/hr, the amount of activity during the photophase was 1125.0±106.9 activity/hr and the amount of activity during the scotophase was 179.5±71.31 activity/hr. The motor activity acrophase was between 12:40 and 14:25.

Visual inspection of actograms showed that the goats' motor activity was mainly diurnal (Fig. 1). These observations are supported by the statistically significant difference in the amount of activity between photophase and scotophase.

ANOVA showed a highly significant effect of time on the serum concentration of tryptophan and serotonin, on each day.

The results obtained during the experimental period indicate the existence of daily rhythms of tryptophan and serotonin serum concentration in the goat, as shown in Fig. 2. Application of the periodic model and the statistical analysis of the Cosinor procedure throughout the time series studied in the different experimental conditions, allowed us to ascertain the periodic pattern of the parameter studied (Table 1). Robust daily rhythmicity was exhibited by tryptophan and serotonin during the two days of experimentation (Table 1). Tryptophan showed a

diurnal acrophase, while serotonin showed a noctural acrophase (Fig. 2).

DISCUSSION

Results obtained in this study show the existence of clear daily rhythms of the parameters studied in goats kept under 12/12 L/D cycle, as previously observed for other physiological parameters in this species (Piccione et al. 2002, 2003, 2007c) and outline a diurnal pattern regarding motor activity and the level of tryptophan in the serum, while the level of serotonin in the serum showed a nocturnal pattern.

Motor activity was prevalent from 08:00 to 18:00, while during the scotophase there are several activity peaks mostly with lower intensity and shorter than during the light period, with several cycles of sleep (Fig. 1).

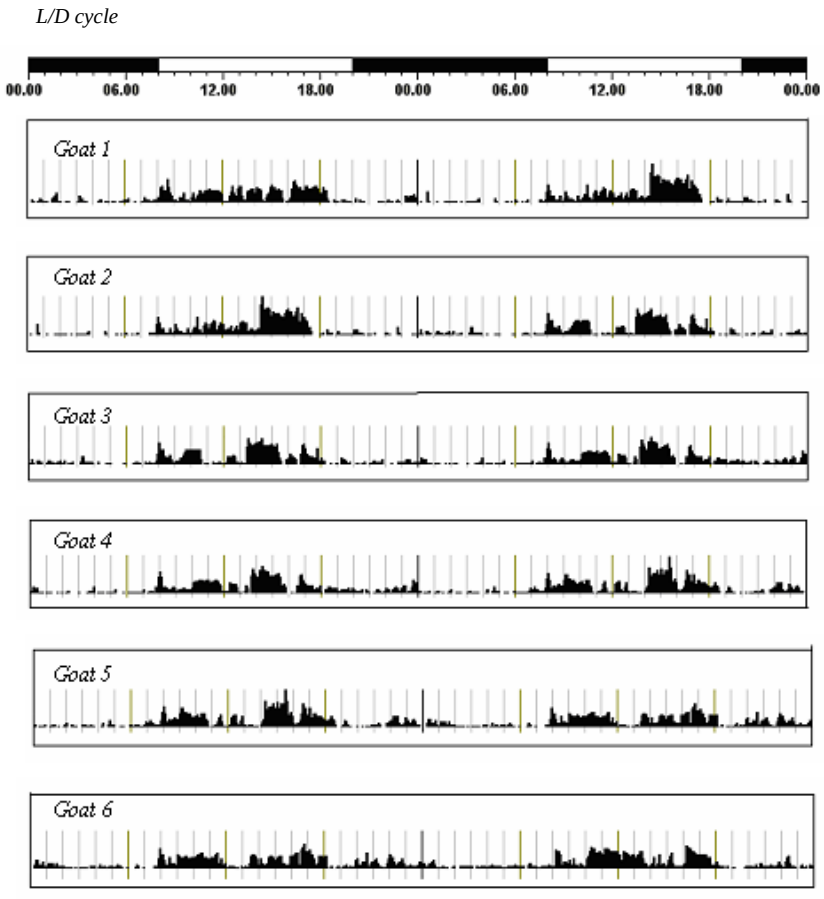

Fig. 1. **Total motor activity recorded in goats exposed to 12/12 L/D cycle.** Each horizontal line is a record of 2 day's activity in each goat. Total activity recorded during consecutive 5-min periods is indicated by vertical black markings. White and black bars at the top of record indicate photophases and scotophases.

Major grazing periods begin near dawn and recur in late afternoon, ending close to sunset (Arnold 1985). During *ad libitum* conditions, water and food consumption occurred throughout the day with a circadian distribution of food and water intake showing peaks at the beginning of the light phase (Rossi et al. 1999). Motor activity acrophase occurred in the middle of the photophase of the

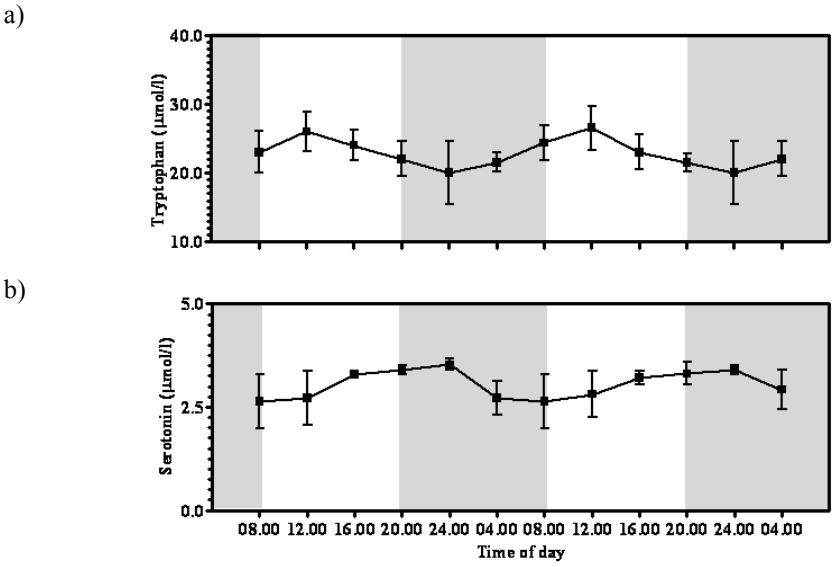

Fig. 2. **Daily rhythms of tryptophan** (a) **and serotonin** (b) **in goats.** Grey bars indicate the dark phase of the 48 h photoperiod.

experimental L/D cycle, as previously observed in the horse (Piccione et al. 2008) and sheep, at least when they were fed *ad libitum* (Piccione et al. 2007b).

Tryptophan and serotonin showed two different serum level trends. Tryptophan acrophase occurred at 12:39 in the day 1 and at 11:17 in the day 2 (Table 1) with a gradual decrease until to reach the lowest values to midnight and then to increase again. Serotonin acrophase occurred at 20:31 in the day 1 and at 21:10 in the day 2 reaching the lowest values at 08:00, at the beginning of the photophase. These confirm that tryptophan hydroxylation is involved in the control of the 5-HT biosynthesis circadian time (Poncet et al. 1993); during the day, the formation of serotonin in the pineal gland is favoured by elevating the uptake of tryptophan, with a gradual decrease of serum level after the diurnal acrophase, whereas at 24:00 other mechanisms, such as induction of enzymes are taking place (Gutierrez et al. 2003). Also the increase of tryptophan before the onset of the scotophase stimulated the melatonin's synchronizing function on the circadian rhythm, regulating the rhythmicity of the motor activity, as previously observed in relation to the immune function (Sánchez et al. 2004, Cubero et al. 2006, Paredes et al. 2007).

In conclusion our results record the daily rhythm of motor activity in goats kept under 12/12 L/D cycle influenced by the endogenous serotoninergic activity controlled by an endogenous clock and showing a substantial impact on neurochemical and behavioural aspects of the circadian response to light.

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