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Daily Rhythms of Total Activity in Rabbits During Different Light/Dark Schedules

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Abstract: The aim of this study was investigated total activity in rabbits subjected to different light/dark schedules. A wide variety of organisms exhibit different circadian rhythms in their behavior and physiology. Circadian rhythms are regulated by internal clocks that are generally entrained primarily by the environmental light/dark (L/D) cycle. Total activity was studied in 5 female rabbits (*Oryctolagus cuniculus*, Blue Vienna breed) subjected to different 12/12 light/dark cycle schedules (Phase 1: light on from 08:00-20:00; Phase 2: light on from 20:00-08:00; Phase 3: light on from 08:00-20:00). General activity of individual animals was monitored continuously by an activity data-logger (Actiwatch, Mini Mitter Co., Bend, OR). The robustness of the activity rhythm was very low in all animals in all stages of the experiment, with all activity concentrated almost exclusively during the dark period.

Key words: Circadian rhythm, total activity, *Oryctolagus cuniculus*

INTRODUCTION

Circadian timekeeping systems play a role in the internal temporal coordination of physiological and behavioral processes of organisms (Jilge, 1993). A wide variety of organisms exhibit daily rhythms of behavioral and physiological changes. These rhythms are mostly generated by an endogenous circadian timing system that is entrained by environmental cues, or zeitgebergs. The light:dark (L:D) cycle is the most potent cue for circadian entrainment in most organisms. Circadian rhythms probably evolved as adaptations that allowed organisms to prepare for relatively predictable environmental changes associated with the day-night cycle (Pittendrigh, 1993). Also, in several species, regulation of circadian timing by behavioral (so-called non-photic) stimuli is well documented. Behavioral arousal stimulated during the middle of the usual rest phase of the circadian rest-activity cycle can induce large phase advance shifts, sufficient to modify the phase angle of entrainment to LD cycles, to greatly accelerate reentrainment to a shifted LD cycle and to stably entrain free-running rhythms in the absence of LD cycles (Mistlberger and Skene, 2004). An alternative method for stimulating activity and arousal in some species is short-term Food Deprivation (FD). However the effects of fasting on the probability or amount of running in a novel wheel during the usual rest period have not been reported (Mistlberger *et al*., 2006).

Rabbits show circadian variation in several behavioral, physiological parameters (Van Den Been and Malpas, 1997) and haematological parameters (Piccione *et al*., 1995). They show a

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nocturnal patter of activity, emerging from the relative safety of burrows at dusk and spending much of the night feeding before retiring in the early hours of the morning (Kennedy *et al*., 1994). Highest values for feeding behavior were observed from the late afternoon to the late night (Jolivet *et al*., 1983), although in one study distinct peaks of feeding activity were found at the beginning and end of the light period (Van Den Been and Malpas, 1997).

On the basis of this knowledge and as a continuation of our research, we studied the total activity in rabbits subjected to different light/dark schedules.

MATERIALS AND METHODS

Five female rabbits (*Oryctolagus cuniculus*, Blue Vienna breed) 12 weeks old with a mean body weight of 2.5 kg were used as experimental subjects in the study that was carried out in 2006 in the Laboratory of Veterinary Chronophysiology, Faculty of Veterinary Medicine, University of Messina (Italy). They were individually housed in metallic cages (90×50 ×35 cm) in a room thermostatically maintained at 21±1°C and were fed rabbit pellets and water *ad libitum*. The experiment was conducted in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences.

The lights in the experimental room (400 Lux) were on for 12 hours each day. For the first 21 days, lights were turned on at 08:00 and turned off at 20:00. On day 22, the L/D cycle was delayed by 12 h (lights on at 20:00 and off at 08:00). On day 43, the L/D cycle returned to the original schedule (lights on at 08:00 and off at 20:00). The experiment was terminated on day 63 (Table 1).

General activity of individual animals was monitored continuously by an activity data-logger (Actiwatch, Mini Mitter Co., Bend, OR) strapped to the animal's neck. 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; 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. 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, UK). Circadian parameters (photic entrainment, length of circadian period, phase-angle of the activity rhythm relative to the zeitgeber phase) in the different experimental conditions were evaluated by visual inspection (Pittendrigh and Daan, 1976a, b) and by means of χ^2 periodogram analysis (Sokolove and Bushell, 1978). Furthermore, average amount of activity (bout of activity/hour) during light and dark phase and Cosine Peak (time of peak activity) were calculating using Actiwatch Activity Analysis 5.06 (Cambridge Neurotechnology Ltd, UK). An index of the robustness (strength) of rhythmicity is provided by the variance accounted for by the cosine model expressed as a percentage of the total variance ($\sigma^2_c \div \sigma^2_t \times 100$).

Table 1: Summer of experimental procedures

Experiment	Days	L/D	Lighting	Procedures
Phase 1	1-21	12:12	08:00-20:00, (400 lux)	Actiwatch recording every 5 min Food *ad libitum*
Phase 2	22-42	12:12	20:00-08:00, (400 lux)	Actiwatch recording every 5 min Food *ad libitum*
Phase 3	43-63	12:12	08:00-20:00, (400 lux)	Actiwatch recording every 5 min Food *ad libitum*

RESULTS

Figure 1 shows the data of the rabbit with the best activity records, indicating a slow shift of the activity rhythm is response to the phase shifts of the L/D cycle.

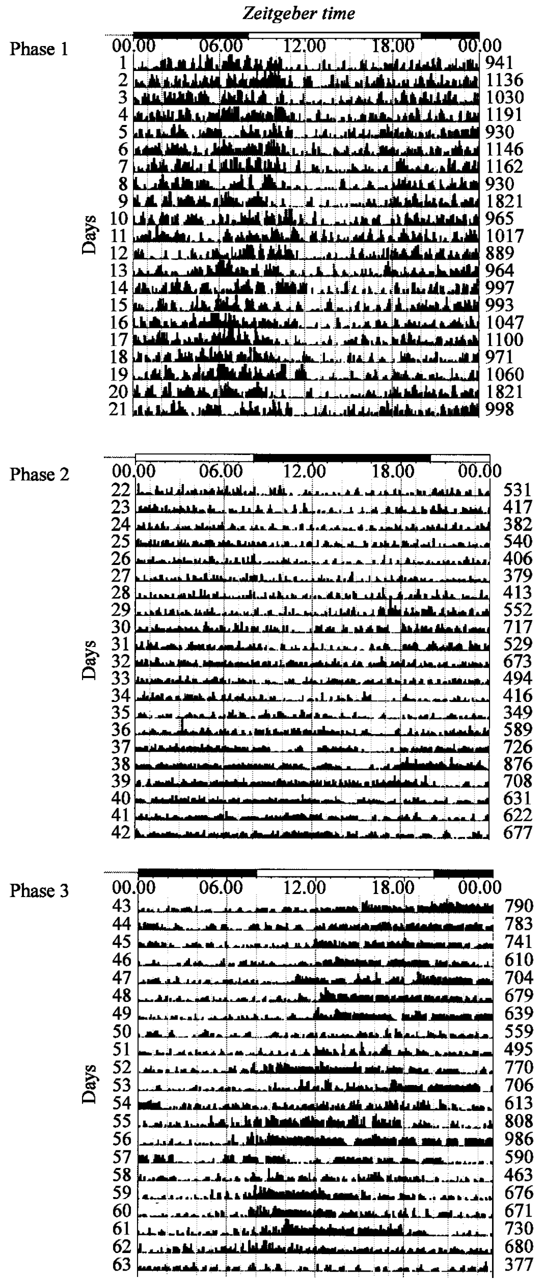

Fig. 1: Total activity record of a rabbit subjected to different 12:12 LD cycles. Each horizontal line is a record of 1 day's activity and consecutive days are plotted one after the other. White and black bars indicate light/dark periods of the 12:12 cycle

During the first 3 weeks (Phase 1), the animal was active mostly during the dark phase of the L/D cycle, although some activity was present at all times, Cosine peak was at 04:50. After the phase delay of the L/D cycle, the activity rhythm seemed to delay slowly but did not attain the same phase angle of entrainment observed prior to the phase shift (Phase 2), Cosine peak was at 04:10. When the L/D cycle was returned to its original time (a 12 h phase advance) (Phase 3) Cosine peak was at 15:15, the activity rhythm seemed to advance slowly but did not attain a stable phase angle of entrainment by the time the experiment was terminated. The robustness of the activity rhythm was very low in all animals in all stages of the experiment.

DISCUSSION

The predominant rhythms in nature are daily rhythms, e.g., those in rest and activity, in body temperature, in concentration of many hormones in bloods and ions in urine, etc. the rhythms are not merely passive responses to the daily alternation of light and darkness as they persist even in non periodic environment (Piccione and Caola 2002). As shown in Fig. 1 the actogram of total activity of a rabbit is relatively clean, with all activity concentrated almost exclusively during the dark period. Rhythms of total activity recorded by actigraphy were not as robust as the rhythms of other species. However, it should be considered that the daily distribution of activity differs not only from species to species but also from individual to individual in the same species (Refinetti, 2006). The low robustness of the total activity rhythm was not a surprising finding. Relatively weak rhythms of total activity and feeding behavior in rabbits have been previously described (Bobbert and Riethoven, 1991; Kennedy *et al*., 1994). Although weak, the activity rhythms of our rabbits were sufficient to characterize their temporal niche as primarily nocturnal. Note that the rabbits, as previously observed in the mouse, a nocturnal animal (Refinetti, 2006), does not run continuously through the dark period; instead, it takes several breaks after an initial long bout of 2-3 h. Rabbits have been previously characterized as primarily nocturnal by laboratory records of activity (Bobbert and Riethoven, 1991; Jilge, 1993), body temperature (Varosi *et al*., 1990; Jilge *et al*., 2001) and blood pressure (Sato *et al*., 1995).

Previous literature indicates that while the rabbit is an endogenously nocturnal animal, external noise or scheduled feeding during the light period can alter its nocturnal activity pattern to one that is predominantly diurnal (Kennedy *et al*., 1994).

In conclusion it is possible to suggest that endogenous timekeeping system, a light-entrainable pacemaker, controls circadian rhythms of total activity in rabbits.

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