

# Now you see me, now you don't: the locomotory activity rhythm of the Asian garden dormouse, *Eliomys melanurus* from Saudi Arabia

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## Abstract

The circadian rhythm of locomotory activity in the Asian garden dormouse, *Eliomys melanurus* from Saudi Arabia was investigated under controlled laboratory conditions. Eleven individuals were subjected to successive light cycles of two weeks. Locomotory activity of the dormouse was confined mainly to the dark phases of the LD and DL cycles. On the LD cycle, the mean percentage of activity was  $95.5 \pm 0.9\%$  during the dark phase. When subjected to constant darkness all animals expressed free running rhythms of locomotor activity (mean  $24\text{h}06 \pm 0\text{h}09$ ). Upon inversion of the LD cycle, activity re-entrained to the new light cycle within 3 days and a percentage of nocturnal activity of  $96.8 \pm 0.7\%$ . When the dark component of the day was lengthened to 8L:16D the active time increased significantly from  $11\text{h}43 \pm 0\text{h}05$  to  $13\text{h}43 \pm 0\text{h}22$ . In contrast, when the dark component was shortened 16L: 8D the active time decreased significantly from that of the 12L:12D to  $7\text{h}58 \pm 0\text{h}04$ . There was no difference in the locomotor activity at 20 and 30°C however, dormice entered torpor at 10°C, being almost completely inactive.

The Asian garden dormouse possesses a circadian rhythm of locomotor activity that entrains strongly to light. The rhythm of locomotor activity occurs predominantly during the dark phase and thus this species can be considered truly nocturnal in habit.

Key words: circadian rhythm, light-dark cycle, locomotor activity, Saudi Arabia, *Eliomys*, dormouse

## Introduction

Circadian rhythms are a common phenomenon in almost all living organisms and are frequently expressed as daily patterns in physiology and behaviour. Circadian rhythms are endogenous, self-sustained and persist when external cues are removed (Meijer & Rietveld 1989). To be of functional value, circadian rhythms need to be synchronised with the external environment, and are therefore adjusted on a daily basis. The light-dark cycle is by far the most predictable and potent environmental time cue and acts as a primary synchronizer or *zeitgeber* of circadian rhythms (Pittendrigh & Minis 1964; Quintero et al. 2003; Skene et al. 1999). In the absence of external cues, circadian rhythms free-run with an intrinsic period close to that of the environmental period. The inherent period of a circadian rhythm is usually species specific, although intra-specific variation may be present (Pittendrigh & Daan 1974; Aschoff 1981; Refinetti 2006).

Circadian timing is organised by a central pacemaker, the suprachiasmatic nucleus (SCN) located in the basal hypothalamus (Stephan & Zucker 1972; Okamura et al. 2002; Froy 2007). Rhythms are generated in the cells of the SCN (Welsch et al 1995), and the basic mechanism of the circadian clock relies on interacting positive and negative transcriptional feedback loops that drive recurrent rhythms in gene expression of the relevant clock genes (Reppert & Weaver, 2002). Biological rhythms allow animals to anticipate predictable changes in the environment to adjust their physiology and behaviour accordingly.

Dormice are small rodents of the family Gliridae, containing some 28 species. The genus *Eliomys* contains three species of garden dormice, occurring in Europe, Africa and Asia. The garden dormouse is endemic to Europe and fairly well researched, however, almost nothing is known about the other two species, the Maghreb and Asian garden dormice.

The Asian garden dormouse has a wide distribution from north-east Africa through to the Middle East, occurring at a variety of altitudes from low lying deserts through to mountainous regions up to 3000m above sea level (Harrison and Bates, 1992; pers. obs.). It inhabits a wide variety of habitats, ranging from semi-desert areas and steppe to high mountains, forested and rocky areas, and cultivated fields (Amori *et al.* 2008, Harrison 1972). The dormouse is an agile climber and well adapted to an arboreal lifestyle but is frequently found on the ground. In some areas they are highly arboreal and in other areas they occur where there are almost no trees (Ognev 1963, Harrison 1972). The Asian dormouse is

described as primarily nocturnal (Novak 1999) and may enter torpor daily (Haim & Rubal 1995).

We examined and describe the basic locomotor activity patterns of the Asian garden dormouse collected near the Raydah protected area in south-western Saudi Arabia. The climate is generally moderate with frequent cloud cover (Birdlife International, 2012; Janin and Besheer, 2003) and vegetation consists of Juniper forests on the higher slopes and more deciduous trees and bushes lower down (BirdLife International 2012).

The aim was to determine whether a circadian rhythm of locomotor activity could entrain to a controlled light-dark cycle and determine the period of the free-running rhythm (*tau*) under constant conditions. In addition, we investigated whether the dormouse could shift its activity according to an inverse in the light cycle to determine whether the transition between light and dark triggers activity or whether they are truly nocturnal. We also examined the effect of long and short days on the activity period, as well as the effect of ambient temperature on activity.

## **Material and methods**

### General

The animals used in this experiment comprised eleven Asian garden dormice (*Eliomys melanurus*), 4 males and 7 females (Mean body mass, mean  $\pm$  SEM:  $89.5 \pm 3.9$ g, range 74-112g). The dormice were collected in the Raydah Protected Area near AbhaCity, in south western Saudi Arabia (18°00'N, 24°46'E). The dormice were trapped with cage live traps (Strauss *et al.* 2008) baited with bread and peanut butter. Traps were set at dusk and were checked at dawn. After capture, the animals were transported to the animal facility at the Department of Zoology, College of Science, KingSaudUniversity, Riyadh, Saudi Arabia where the experimental procedures were carried out. The experimental protocol was evaluated by the Animal Ethics Committee of the University of Pretoria, ethics clearance number EC015-12.

The dormice were housed individually in plastic terraria (50x40 x40cm) and provided with wood shavings and tissue paper as nesting material. Food and water was provided *ad libitum*, and a few drops of multivitamins were added to the drinking water. Animals were fed on pelleted poultry food from the Arabian Agricultural Services Company (Arasco, Riyadh, Saudi Arabia) every 14 days at the end of the cycle. The diet was supplemented with carrots and cucumber when light cycles were changed. During the feeding times the general health

and condition of the animals was monitored. The experimental room was temperature controlled at  $25 \pm 1^\circ\text{C}$  and the room was light controlled which enabled us to create different light regimes for the experimental periods.

#### Experimental procedures

Animals were maintained on a 12L:12D cycle (6:00 – 18:00L) for 14 days to determine how well they entrain to light cycles. This was followed by a 14 day DD cycle in order to determine whether the dormice possess endogenous free running rhythms of locomotor activity. Subsequently the lights were switched back to the original 12L:12D cycle to re-entrain the animals, after which the light cycle was inversed to investigate whether and how fast the animals change their activity according to the new light cycle.

Next the dormice were subjected to light cycles of different lengths at an ambient temperature of  $25^\circ\text{C}$ . First animals were acclimated to a 12L:12D light cycle for a week, following a two week period of 8L:16D simulating a short day, and a two week period of 16L:8D simulating a long day.

Finally, animals were returned to a LD light cycle and temperature was changed every 14 days. Temperature regimes were in the following sequence:  $30^\circ\text{C}$ ,  $20^\circ\text{C}$  and  $10^\circ\text{C}$ .

#### Activity measurements

An infrared captor (Quest PIR internal passive infrared detector; Elite security products (ESP), Electronic lines, UK) was fitted above each of the cages in which the animal was housed. These sensors were placed in the middle of each cage to detect any movement that the animal made in any area of the cage. A reading was taken once every minute and the activity measurements were captured by the program Vital View on a computer system (Vital View<sup>TM</sup>, Minimitter Co., Inc., Sunriver, OR, USA; [www.minimitter.com](http://www.minimitter.com)).

#### Data analyses

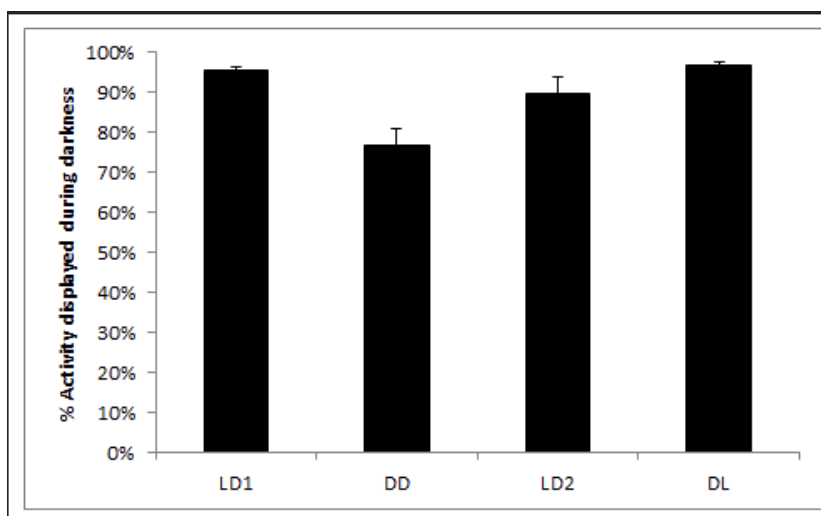
Activity was depicted as double plotted actograms with Actview Biological Rhythm Analyses 1.2 software (Minimitter Co., Inc., Sunriver, OR, USA; [www.minimitter.com](http://www.minimitter.com)) to visualise activity patterns. Clocklab was used for all activity analyses (ClockLab<sup>TM</sup>, Actimetrics, Evanston, IL, U.S.A.). To determine statistical differences between different light cycles, Statistica 10.0 (© StatSoft, Inc. 1984-2011) was employed. Due to small sample sizes, non-parametric statistics were used for all calculations.

## Results

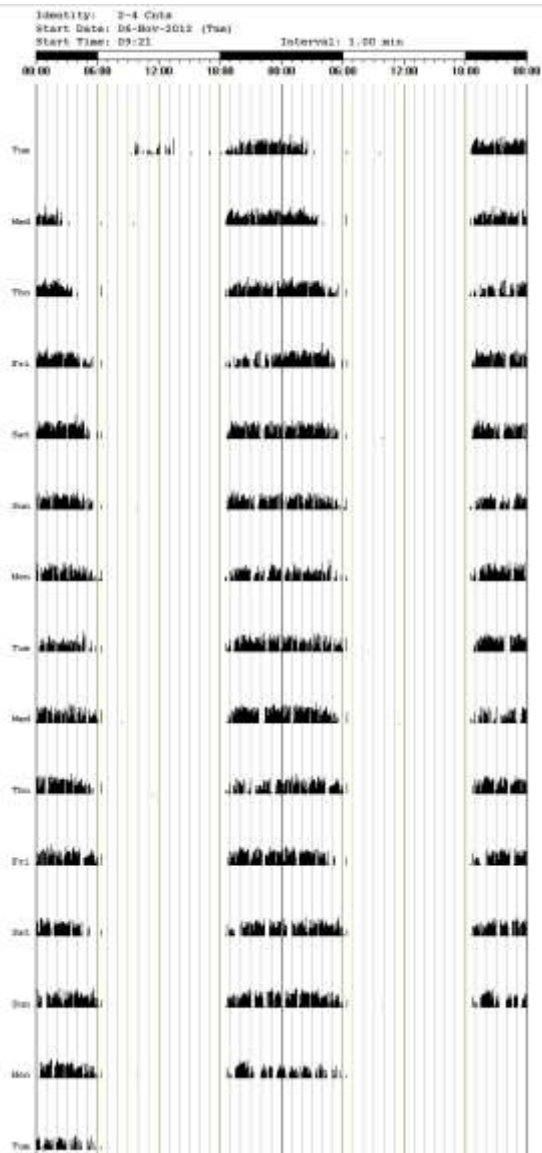
### 12L:12D

All eleven dormice displayed entrainment of their locomotor activity to a 12L:12D light cycle. All individuals were active exclusively during the dark phase of the light cycle ( $n=11$ , mean =  $95.5 \pm 0.9\%$ ), and the activity was mostly continuous. The peak activity time for most animals occurred about two hours after onset of the dark phase ( $n=9$ , mean =  $19\text{h}57 \pm 0\text{h}08$ ).

All but one animal displayed distinct onsets, and three animals exhibited clear offsets. The mean phase angle for the onsets was  $19\text{h}14 \pm 0\text{h}04$  while the mean phase angle for offsets was  $6\text{h}24 \pm 0\text{h}04$  (Figure 1, 2).



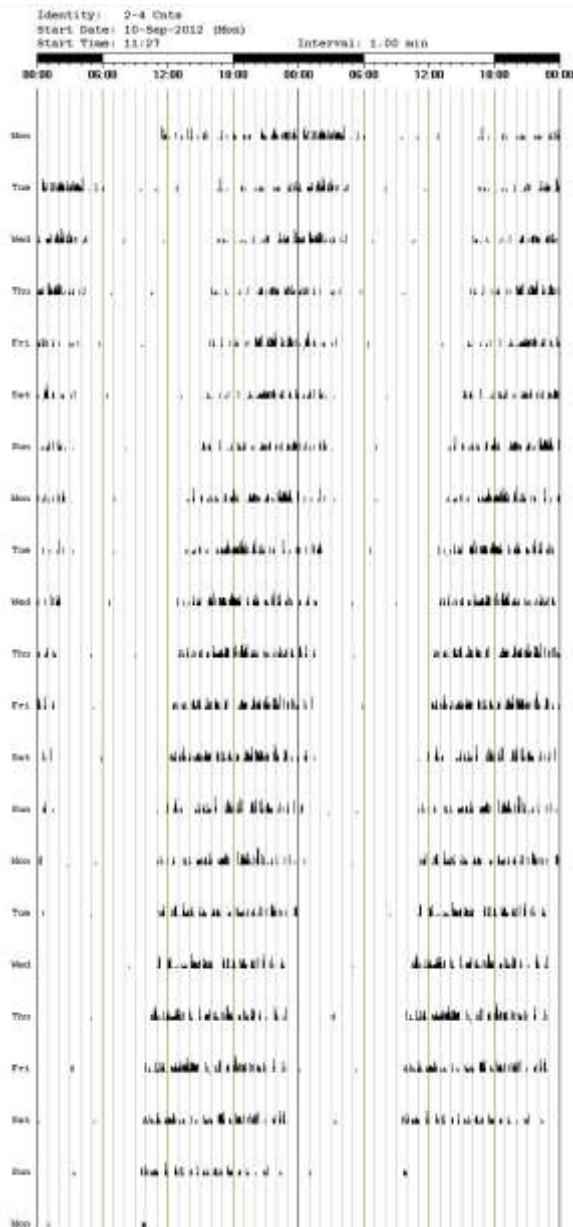
**Figure 1.** Mean percentage of night time activity ( $\pm$  SEM) of *E.melanurus* during the different light cycles.



**Figure 2.** A double plotted actogram of a Asian gardendormouse displaying activity well entrained to the 12L:12D light cycle. Black bars on top of the actogram illustrate dark periods, and consecutive days are on the Y-axis.

## DD

All eleven dormice showed circadian rhythmicity, six dormice displayed circadian rhythms shorter than 24 hours, while the others were longer than 24h hours. The mean period was  $24\text{h}06 \pm 0\text{h}09$ , ( $n=11$ , range  $23\text{h}57\text{-}25\text{h}02$ ). The mean percentage of activity during the subjective night was  $77 \pm 4.1\%$ , which is a significant decrease in nocturnal activity compared to the first LD cycle (Wilcoxon matched paired test  $n = 11$ ;  $p = 0.003$ )(Figure 1, 3).

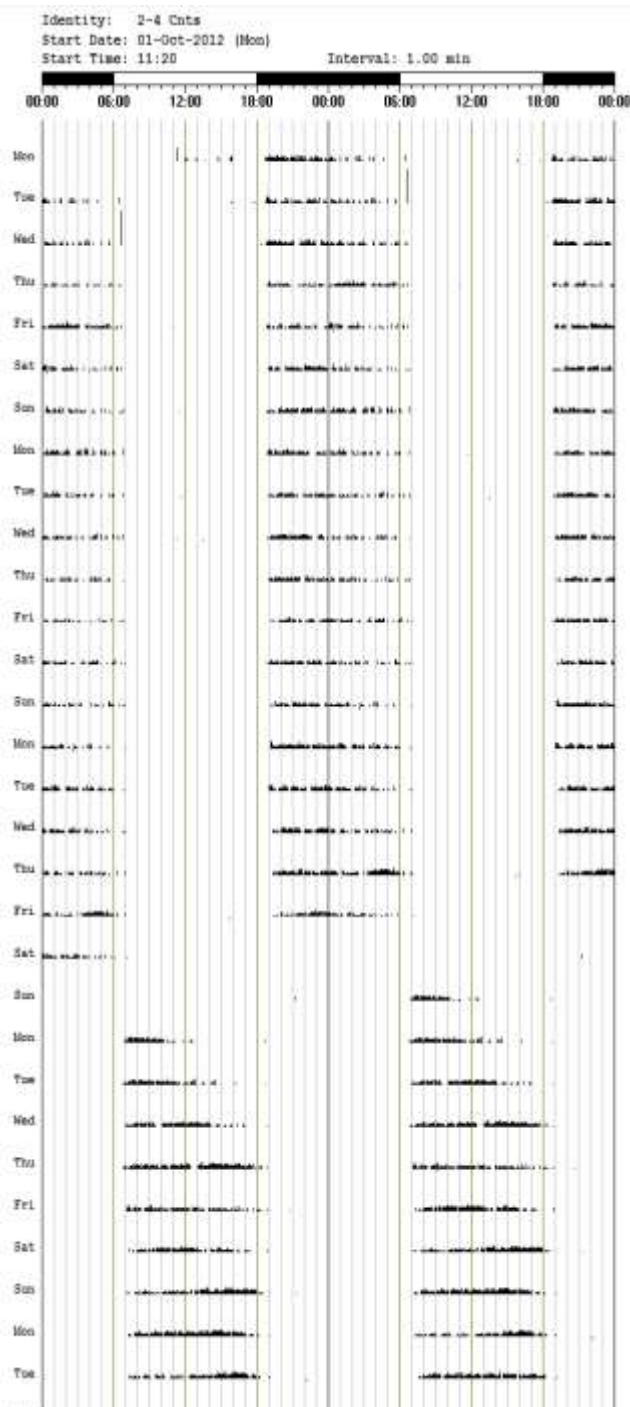


**Figure 3.** (a) An example of a free running rhythm with a period slightly shorter than 24h. (b) Periodogram of the same individual showing the period of the rhythm at 23.98h.

### LD and inverted LD

All dormice inverted their activity when the light and dark phases were inverted. After inverting the light cycles, re-entrainment of the onset of activity to the new cycle took place relatively quickly and masking effects were not very apparent and full entrainment of the activity rhythm varied between 2 and 4 days, with a mean time of 3.3 days. The mean percentage of activity during the dark phase of DL was  $96.8 \pm 0.7\%$ . The percentage of activity displayed during the dark phases of LD2 and DL was significantly different (89.6 vs

96.8%) from one another (Wilcoxon matched paired test  $n=10$ ,  $Z = 2.59$ ;  $T = 2$ ;  $p = 0.009$ )(Figure 1, 4).



**Figure 4.** An actogram of a dormouse demonstrating activity when the light cycle was inverted from LD to DL. Re-entrainment is fast and masking of the activity is not apparent. Bars on top of the actogram depict light and dark periods, and consecutive days are on the Y-axis.



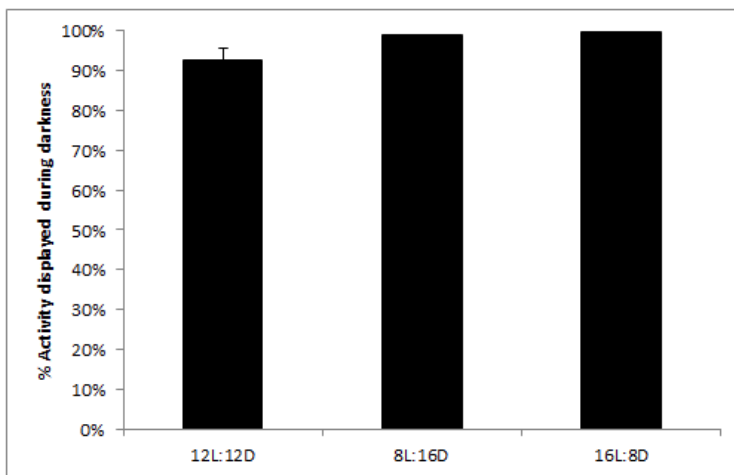
There was not a significant difference in the percentage of activity during the dark phase of LD1 and LD2 in the dormouse (Wilcoxon matched paired test  $n=10$ ,  $Z = 1.07$ ;  $T =17$ ;  $p = 0.28$ ).

Long (16L:8D) and short (8L:16D) days

All but one of the ten dormice exhibited more than 90% activity during the dark phase of the 12L:12D light cycle ( $n=10$ , mean =  $92.7 \pm 3.0\%$ ). The mean time activity ( $\alpha$ ) is  $11h43 \pm 0h05$ .

When the dark component of the day was lengthened to 8L:16D, the mean percentage of activity during the dark increased to  $98.8 \pm 0.2 \%$  ( $n=10$ ). This is a significant increase in dark time activity from 12L:12D (Wilcoxon matched paired test  $n=11$ ,  $Z = 2.13$ ;  $T =9$ ;  $p = 0.03$ ). The mean  $\alpha$  during the night is expanded along with the longer night to  $13h43 \pm 0h22$ , this is significantly longer than during 12L:12D (Wilcoxon matched paired test  $n=11$ ,  $Z = 2.9$ ;  $T =0$ ;  $p = 0.003$ )(Figure 5, 6).

When the dark phase length was decreased to 16L:8D, no decrease in the percentage of night time activity was detected, infact, it increased slightly ( $n=11$ , mean  $99.7 \pm 0.1\%$ ), Wilcoxon matched paired test  $n=11$ ,  $Z = 2.9$ ;  $T =0$ ;  $p = 0.003$ )(Figure 5, 6).



**Figure 5.** Mean percentage of activity ( $\pm$  SEM) during the dark phase of light cycles with different lengths.



**Figure 6.** A double plotted actogram demonstrating the expanded activity time with longer dark periods (8L\_16D) and reduction of active time with shorter dark periods (16L:8D). Bars on the top of the actogram depict 12L:12D light and dark times and consecutive days are on the Y-axis.

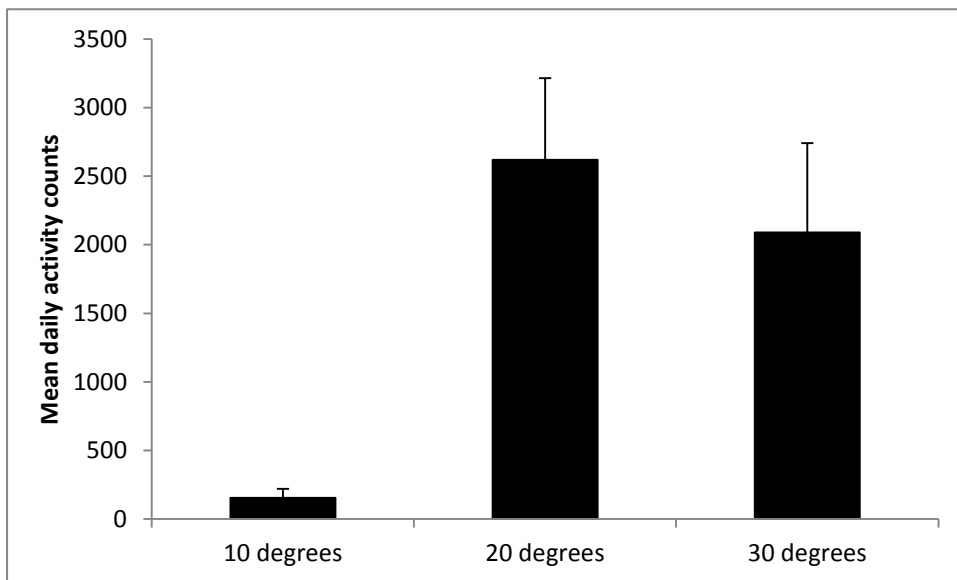
The mean  $\alpha$  for activity decreased as the night was shortened, and was significantly less than for 12L:12D (mean = 7h58  $\pm$  0h04; Wilcoxon matched paired test n=11, Z = 2.9; T =0; p = 0.007).

#### Temperature variation on a 12L:12D light cycle

All animals maintained nocturnal activity patterns during all three of the temperature variations. The mean percentage of activity during the night was 93.6 $\pm$ 3.8% at 10°C, 94.9 $\pm$ 0.97% at 20°C and 98.64 $\pm$ 0.52% at 30 °C. This is not significant between 10°C and

20°C (Wilcoxon matched paired test n=11, Z = 0.53; T =27; p = 0.59) and 10°C and 30°C (Wilcoxon matched paired test n=11, Z = 1.69; T =14; p = 0.09), but the amount of nocturnal activity was significantly higher at 30°C compared to 20°C (Wilcoxon matched paired test n=11, Z = 2.9; T =0; p = 0.003).

Mean total daily activity counts was 155±92.4 at 10°C, 2117.5±837.2 at 20°C and 2090.5±922 at 30 °C. This was not significantly different between 20°C and 30°C (Wilcoxon matched paired test n=11, Z = 0.71; T =25; p = 0.48), but there was a significant difference between both 10°C and 20°C (Wilcoxon matched paired test n=11, Z = 2.9; T =0; p = 0.003) and 10°C and 30°C (Wilcoxon matched paired test n=11, Z = 2.9; T =0; p = 0.003).



**Figure 7.** Mean ( $\pm$ SEM) level of daily activity of *E.melanurus* at three different ambient temperatures

## Discussion

In controlled conditions, the Asian garden dormouse shows almost exclusively nocturnal activity, commencing its activity after the transition from light to dark and terminating it before the next light cycle. All dormice were captured during the night, indicating that they are nocturnal in their natural environment as well. In addition, the Asian garden dormouse shows a daily rhythm of  $VO_2$  and  $T_b$  consistent with that for a nocturnal species, with increased oxygen consumption and raised body temperature during the dark period (Haim & Rubal 1995). Predominantly nocturnal activity has also been demonstrated in other dormice

species, both in field and laboratory based studies (Daan 1973, Kastenmayer et al 2010, Rodolfi 1994).

Asian garden dormice show very distinct onsets of activity with marked activity shortly after the onset. Activity offsets were not as clear cut, implying that the most important entraining agent is the transition period from light to dark. A dual oscillator system has been proposed to explain differential responses to on and offsets of activity in rodents (Daan et al. 2001), in this case the well-defined onsets suggest that the E oscillator has a stronger link to locomotor activity than the M oscillator.

As far as we are aware, free-running rhythms have so far not been described for dormice. When maintained under constant darkness, all of the dormice showed an endogenous rhythm of locomotor activity that drifted slightly relative to solar time. About half of the dormice exhibited free running rhythms shorter than 24 hours, while the others were longer than 24 hours. Free-running rhythms are frequently shorter than 24h in nocturnal animals (Jud *et al.* 2005) although large intra-species variability is not uncommon. The length of the free-running period does not seem to have any specific relevance as the free-running period of rodents has been shown to be a plastic property, and can be influenced by environmental and experimental conditions (Aschoff 1979, Weber & Hohn 2005, Weisgerber *et al.* 1997).

Dormice revealed an immediate response in the onset of their activity to an inverse in the light cycle. The re-entrainment of activity was by means of a phase delay and took place relatively fast. A masking effect of activity is clearly visible during the light phase of the DL cycle, emphasizing that these animals are strictly nocturnal. The rate of re-entrainment depends on a number of internal and external factors such as the strength of the *zeitgeber*, the period and phase of response curve of the pacemaker and experimental protocol (Aschoff *et al.* 1975, Pittendrigh & Daan 1976), which renders it challenging to compare between different studies and species.

The dormouse responded to a change in photoperiod exerted on it under laboratory conditions, under a short day 8L:16D the amount of activity restricted to the dark period peaked at around 96% of all activity, whereas under a long day, 16L:8D activity devoted to the dark period increased to 99%. Dormice inhabiting temperate regions hibernate during winter, thus it may be interesting to maintain these animals on a short photoperiod for longer periods of time to establish whether hibernation is triggered primarily by decreasing

temperature, or whether photoperiod plays a role in the onset of fat accumulation and other physiological changes associated with hibernation.

As mentioned before, hibernation is common in dormice occurring in temperate regions. By varying ambient temperature we hoped to observe a change in activity levels in the dormice. At 20 and 30 °C, the amount of activity exhibited by the dormouse is similar, although the activity distribution is shifted slightly, at 30 °C dormice displayed a higher percentage of activity during the night. However, at an ambient temperature of 10 °C, Asian garden dormice were virtually inactive. Dormice have been reported to be torpid on several consecutive days during winter (Haim & Rubal 1995). Hibernation is not a continuous process, and the length of the torpor bouts is influenced by environmental temperature (Daan 1973).

This study provides the first description of detailed locomotor activity patterns for dormice. Asian garden dormice show strictly nocturnal activity, and entrain their activity to light cycles. The locomotor activity responds to photoperiod and an ambient temperature of 10 °C is low enough to induce a state of torpor.

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