

## Circadian rhythm of locomotor activity in the four-striped field mouse, *Rhabdomys pumilio*: A diurnal African rodent

Desiree M. Schumann<sup>a</sup>, Howard M. Cooper<sup>b</sup>, Margaretha D. Hofmeyr<sup>a</sup>, Nigel C. Bennett<sup>c,\*</sup>

<sup>a</sup>Department of Biodiversity and Conservation Biology, University of the Western Cape, Bellville, South Africa

<sup>b</sup>Cerveau et Vision, Unit 371, INSERM, Avenue du Doyen, Lepine, Bron, France

<sup>c</sup>Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa

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### Abstract

Although humans are diurnal in behaviour, animal models used for the study of circadian rhythms are mainly restricted to nocturnal rodents. This study focussed on the circadian behaviour of a rodent from South Africa that has a preference for daylight, the four-striped field mouse, *Rhabdomys pumilio*. In order to characterise the behavioural pattern of daily activity, locomotor rhythms were studied under different light regimes using an automated data recording system. Under conditions of natural daylight, which include dawn and dusk transitions, *R. pumilio* exhibited activity restricted to the daytime period. Activity was concentrated around morning and evening with a decrease during mid-day. A similar diurnal preference pattern of behaviour was recorded under a light–dark cycle of artificial illumination. Under conditions of constant darkness, the four-striped field mouse exhibited a free-running circadian rhythm of locomotor activity with activity concentrated during the subjective day. Free-running rhythms varied greatly between individuals, from slightly less to slightly more than 24 h (range=23.10 to 24.80 h). Under conditions of constant light, the mice were more active during subjective day, but the free-running rhythm in all individuals was consistently longer than 24 h (range=24.30 to 24.79 h).

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### 1. Introduction

Almost all species are adapted to entrain their behaviour on a daily or 24-h basis with the main zeitgeber (environmental cue) being light. Animals can therefore be characterised as nocturnal, diurnal or crepuscular based on the proportion of their activity occurring during the night, day or twilight times, respectively. The mammalian circadian system has been extensively investigated in nocturnal mammals and, while these studies are also applied to diurnal mammals which also include humans, it is not known whether they provide an adequate model. The neural basis at which nocturnal and diurnal mammals are differentiated is unknown; however, it is thought that neural activity in areas other than the SCN may be

involved [1,2] since the SCN shows a higher firing rate during the day than night in both nocturnal and diurnal mammals [3,4]. In addition, hypothalamic multiple unit activity outside the SCN is out of phase with the endogenous activity of the SCN but in-phase with the activity pattern in nocturnal mammals, whereas in diurnal mammals it is in-phase both with the SCN and with the activity pattern. It has also been suggested that diurnality or nocturnality in a species may be controlled by processes in the intergeniculate leaflet. Whereas inter-specific differences on the other hand may be controlled by the lower sub-paraventricular zone [5].

Nocturnal and diurnal mammals also differ in retinal structure and the relative number of rods and cones and this is related to the period of activity. The European ground squirrel is active throughout the day, activity starting after dawn and ending before dusk, it has a high percentage of cones in relation to rods [6–8]. Hamsters, in contrast, are

\* Corresponding author. Tel.: +27 12 4202538; fax: +27 12 3625242.

E-mail address: ncbennett@zoology.up.ac.za (N.C. Bennett).

active at night and have a high percentage of rods in relation to cones [9]. Nocturnal mammals thus are more sensitive to light than diurnal mammals as a result of their photoreceptor composition.

The distinction between nocturnality and diurnality is not always fixed or clear-cut. A switch in activity patterns from diurnal to nocturnal may occur in certain mammals when exposed to running wheels, as occurs in *Octodon degus* [10,11], and *Arvicanthis niloticus* [12], or from diurnal to nocturnal due to inter-specific competition, as is the case with the golden spiny mouse [13]. Thus, although light is a major entraining agent, it would appear that activity patterns can be masked by other environmental factors such as competition for resource and food territories.

The four-striped field mouse is distributed discontinuously from Southern Africa (South Africa, Angola, Botswana, Zimbabwe and Malawi) to East Africa (Kenya, Tanzania and Uganda) [14–16]. They live above ground in heavily vegetated areas and their nest sites are characterised by runways, which lead from one bush to another. The mouse feeds predominantly on grass seeds. They are grey–brown in colour, with four distinctive black stripes on their back, are approximately 10–14 cm in length and weigh 40–70 g. The breeding season is from September to April in the southern hemisphere and the gestation period ranges from 23 to 25 days [6]. Although there have been conflicting reports about the activity patterns of *R. pumilio*, most authors consider them diurnal [14,15,17].

*Rhabdomys pumilio* is a murid rodent that has been hypothesized to be diurnal based upon field observation [14–16]. In this study, the activity patterns for *R. pumilio* were quantitatively determined as well as the effects of different light schedules on the locomotory activity. Six males were placed under natural lighting conditions to determine the effects of twilight on the activity pattern. At the start of the experiment, the animals were maintained without running wheels, since in certain mammals there is a change in activity when exposed to this condition. A further six males were maintained on a square wave lighting schedule. When an animal's rhythm is entrained, its system adopts a specific phase relationship with the zeitgeber. If placed in constant conditions, the rhythm persists with a free-running period,  $\tau$ , close to 24 h [18].  $\tau$ , the free-running period during constant darkness and light, was calculated to determine the free-running period of *R. pumilio*. The free-running period under constant light and dark was undertaken in this study to assess how it matched up to the general predictions proposed for mammals in general as predicted by Daan, Pittendrigh and Aschoff [10,19]. The start of subjective day was regarded as equivalent to activity onset according to the definition of Pittendrigh [20] and the end of subjective day (CT12) corresponded to  $\tau$  divided by two. Pittendrigh [21] stated that the beginning of subjective day corresponds to

'that point in a constant dark rhythm whose normal phase in a light–dark cycle coincides with the dark–light transition'.

In this study, we investigated the onset and offset of activity and the period of the locomotory activity pattern of male four striped field mice under natural twilight and a square wave lighting regime.

## 2. Materials and methods

### 2.1. Experimental procedures

Twelve male *R. pumilio* were live-trapped with Sherman traps in the Cape Flats Nature Reserve, Cape Town, South Africa (33°55S, 18°22E). The animals were individually housed in plastic cages (60 × 45 × 35 cm) equipped with running wheels (17 cm), nests and infra-red captors in a light-controlled room. The running wheels were placed directly in-line with the infra-red captors, so that activity within the wheel as well as within the cage could be recorded. The animals were fed at varying times, every second day, and were provided with water ad libitum. For the initial 12:12 LD cycle (LD1), six of the males were placed outside (in natural conditions) in individual cages and six were placed in a light-controlled room supplied with fluorescent tubes emitting light levels of approximately 500 lx where non-photoc stimuli such as temperature and relative humidity were controlled. This was to determine the effects of twilight on the activity patterns of *R. pumilio*. Once entrainment was stable with the running wheels present (14 days), the animals were all placed in the light-controlled room in constant dark (DD) for 34 days. The animals were entrained on a 12:12 LD cycle (LD2) for 15 days after being in constant conditions. Finally, we exposed *R. pumilio* to constant light (LL) for 32 days followed by a 12:12 LD cycle (LD3). Lights were automatically switched on at 6:00 h and switched off at 18:00 h for all the artificial light cycles. Activity was recorded every minute by an infra-red captor connected to a mini-mitter system (Vitalview).

Animals maintained under civil twilight could not be controlled for non-photoc environmental stimuli such as changes in temperature and humidity.

### 2.2. Data analysis

Actograms were produced using Actiwatch software. Activity parameters were estimated using Clocklab software and the running mean method [20]. All measurements during LD1 started prior to the running wheel being installed. The onset of activity for an animal was considered significant when the animal was active continuously for longer than 10 min and activity offset occurred when the animal was not continuously active for 20 min. Phase-angle difference and percentage diurnality was calculated during

the 12L:12D cycles. Phase-angle difference was calculated by finding the difference between activity onset/offset and lights on or lights off, respectively. To determine whether the animal preferred light to dark, activity was summed for the 12 h of light and 12 h of dark. Percentage activity during the light phase was equal to the activity during the 12 h of light divided by the total activity (i.e. activity during 12 h of light added to activity during 12 h of dark) multiplied by 100. Percentage diurnality during the subjective day of DD and LL was calculated in the same manner. Percentage activity during subjective day was equal to the ratio of activity during subjective day and total activity. The duration of the active period ( $\alpha$ ) during the 12:12 LD cycle, DD and LL as well as the period,  $\tau$ , of the endogenous clock during LL and DD was calculated.  $\alpha$  started with activity onset and ended with activity offset. Results are calculated as mean  $\pm$  standard error.

### 2.3. Statistical analysis

The active periods during constant dark, LD2 and constant light were compared using a one factor repeat measures ANOVA. A *t*-test was performed to test the difference in activity onset and offset between animals in a square light–dark cycle and animals in a twilight cycle.

## 3. Results

Actograms for *R. pumilio* during LD1 for animals kept in a square light–dark cycle or animals kept under natural conditions are presented in Fig. 1. It is clear that *R. pumilio* was able to entrain to a light–dark cycle in both conditions and exhibited a mainly diurnal pattern of locomotor activity. Activity onset is clear and occurred approximately at the same time each day within each group. In the 12L:12D group, activity began at 4:42  $\pm$  0:17 h, prior to lights on at 6:00 h, and at 5:42  $\pm$  0:12 h in the group exposed to the natural light cycle. Activity offset occurred at 18:48  $\pm$  0:09 h, after lights off in the 12L:12D group and at 20:23  $\pm$  0:31 h in natural light conditions. Since light varies continuously in natural conditions, the onsets and offsets of activity in relation to ‘lights on’ cannot be defined in the same way, but will be discussed below.

All *R. pumilio* showed a bimodal activity pattern with a clear peak at dawn and dusk, separated by a rest period or decrease in activity during the middle of the day (Fig. 1). Morning and evening peaks were distinct in all animals under both lighting conditions. In the LD group, the morning peak occurred at 6:28  $\pm$  0:15 h, after lights on, and at 17:17  $\pm$  0:21 h prior to lights off. In the natural light cycle, morning and evening peaks were at 6:41  $\pm$  0:12 h and 18:20  $\pm$  0:16 h, respectively. In both, the average difference between the two peaks of activity was less than 12 h (LD=10.80, natural light=11.63). The length of the rest period varied from 5 to 8 h depending on the individual.

In this initial phase of the experiment, the animals were maintained approximately 2 weeks in their cages prior to the introduction of a running wheel. Qualitative analysis revealed no major differences in the activity patterns before and after the presence of the running wheel. The only effect observed was in one or two animals (see Fig. 1, animal no. 6), where the onset and offset of activity became more precise and the amplitude of the activity increased after the running wheel was introduced.

The patterns of activity exhibited by all the animals on the three LD cycles are presented in Fig. 2. Most of the animals displayed a higher percentage of activity during the light phase than during the dark phase of all three LD cycles. However, three animals (animal nos. 2, 3 and 8) showed a slightly higher percentage of activity during the dark phase during one of the three LD cycles. One of the animals (animal no. 7) showed more activity during the dark phase of all three LD cycles. Even though the majority of the activity was during the dark phase, this animal was nevertheless mainly ‘crepuscular’ in that activity occurred at the light–dark transitions (Fig. 3).

Considering periods LD2 and LD3, when all the animals were in the same conditions, the average time of onset of activity was 5:12  $\pm$  0:09 h and the average offsets occurred at 19:02  $\pm$  0:15 h. The length of the active period,  $\alpha$ , changed from one 12L:12D cycle to another, though no systematic pattern in the individual changes was observed. There are more variations in  $\alpha$  during LD2 (13.93  $\pm$  0.48) that followed DD, than during LD3 (13.75  $\pm$  0.23), which occurred after the animals were in LL. Changes in  $\alpha$  appear to be due to variability in the activity offset rather than the activity onsets. As in the earlier phase of the experiment, morning and evening peaks were clear in all the animals (M peak=6:19  $\pm$  0:11 h and E peak=18:01  $\pm$  0:11 h).

In constant light and constant dark conditions, *R. pumilio* showed distinct free-running periods (Fig. 4). There were large inter-individual variations in  $\tau$  in constant dark ranging from 23.05 to 24.80 (24.05  $\pm$  0.15, Fig. 5). In constant light, there was less variability in  $\tau$  (24.30 to 24.78) that increased to 24.43  $\pm$  0.02. Animals with  $\tau_{DD} < 24$  h showed an increase in  $\tau$  during LL, but animals with  $\tau_{DD} > 24$  h maintained their period or showed a slight decrease in  $\tau$  during LL.  $\alpha$  changes significantly when the lighting conditions change from DD (15.48  $\pm$  0.93) to 12:12 LD (13.93  $\pm$  0.48) to LL (16.52  $\pm$  0.75). There is a significant difference between the length of the activity periods during DD and LD2 ( $p=0.01$ ), LD2 and LL ( $p<0.01$ ), and DD and LL ( $p<0.01$ ).

Interestingly, the basic pattern of activity, regardless of the changes in  $\tau$  or  $\alpha$ , was conserved during the constant light or dark conditions. Eighty-three percent of the mice (10 of 12) maintained distinct morning and evening peaks as well as the mid-day rest period (see Fig. 4). The time between the morning and evening peaks was shorter in DD (10.85) than in LL (12.97). No splitting of activity was seen

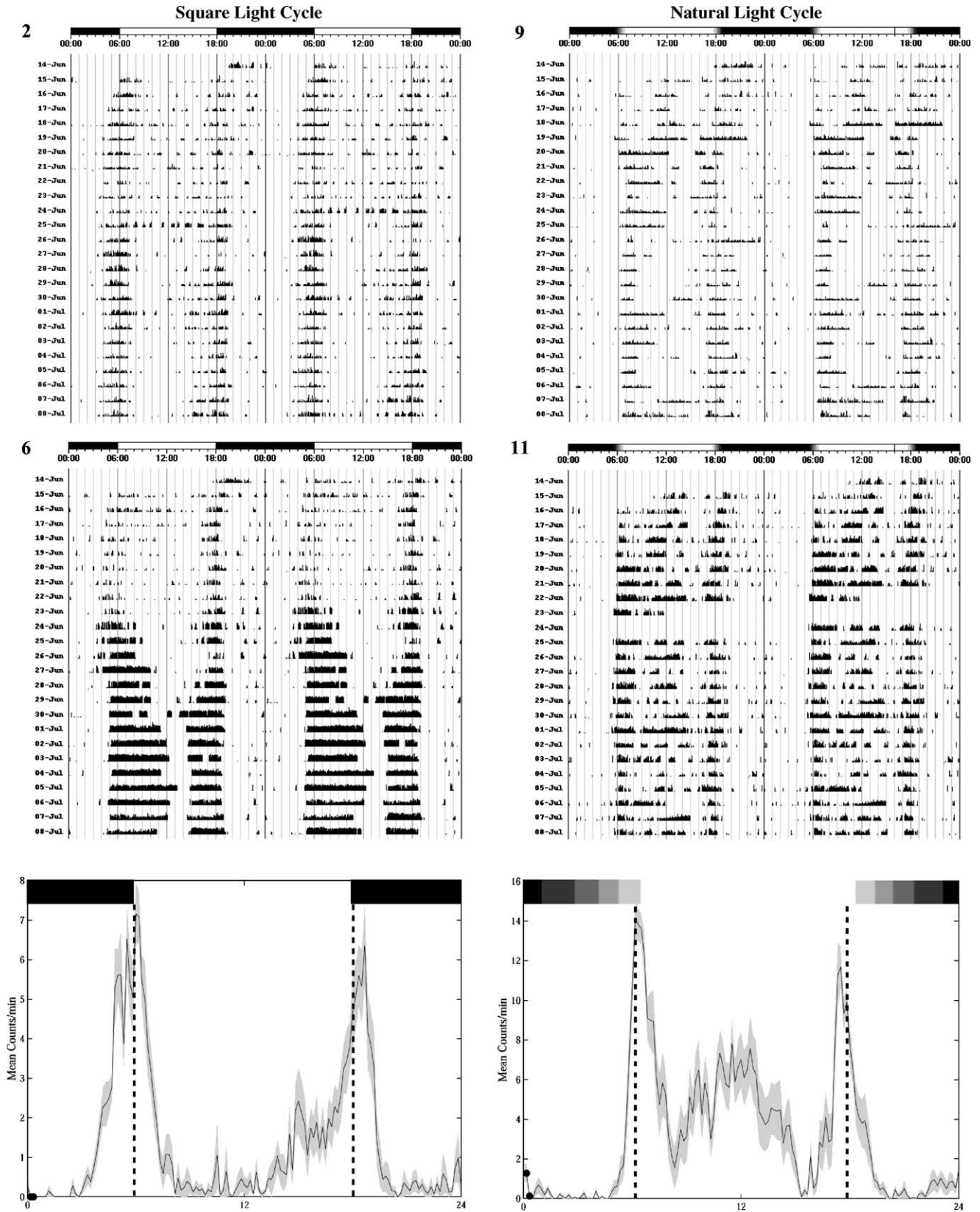


Fig. 1. Representative actograms for *R. pumilio* showing locomotory activity during a 12:12 LD cycle (LD1). The top two actograms are of mice kept on a square light cycle (animal nos. 2 and 6) and the bottom two actograms are of mice kept in natural conditions (animal nos. 9 and 11). The activity profiles are for animal nos. 2 and 11 during LD1. Two peaks of activity are evident with a decrease in activity during the middle of the day. The dashed lines on the activity profile indicate lights on and off for square light cycle and daylight for the animals maintained under natural conditions.

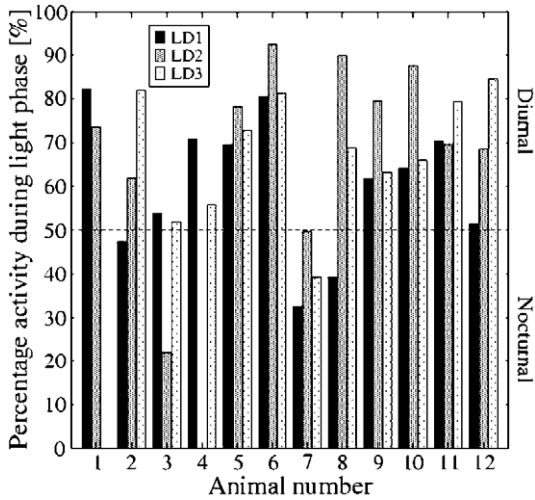


Fig. 2. Activity of each individual during the light phase expressed as a percentage of its total activity. Activity of more than 50% during the light phase indicates that the animal is diurnal because the animal is more active during the light phase than during the dark phase.

in *R. pumilio*. Two animals showed ultradian rhythmicity 10–14 days after being placed in LL.

In constant dark or light conditions, the amount of activity during subjective day and subjective night is dependent on the method of calculation. The beginning of

subjective day (CT0) is usually regarded as the time of activity onset for a diurnal animal. Most animals were more active during subjective day (CT0–CT12) in both constant dark and constant light (Fig. 6). Once again, it is noteworthy that only animal no. 7 exhibited ‘nocturnal’ behaviour in both DD and LL as well as in LD (Figs. 3 and 6). In contrast, animal nos. 5 and 11 were slightly nocturnal in LL, but clearly diurnal in all LD conditions.

However, the onset of activity of *R. pumilio* in LD conditions occurs prior to lights on. Thus, using the onset of activity to define the beginning of subjective day appears incorrect, if subjective day is considered as the period during which light would be present in relation to the animal’s circadian time. This corresponds to Pittendrigh’s [21] definition of subjective day ‘that point in a constant dark rhythm whose normal phase in a light–dark cycle coincides with the dark–light transition’. We thus used the average phase-angle difference between onset of activity and lights on during LD2 and LD3 for each animal (48 min) to calculate the beginning of subjective day. Using this method, the percent of activity that occurs during subjective day is essentially identical to that calculated using the method in the preceding paragraph.

The same problem for defining the beginning and end of day applies to the animals under natural lighting conditions

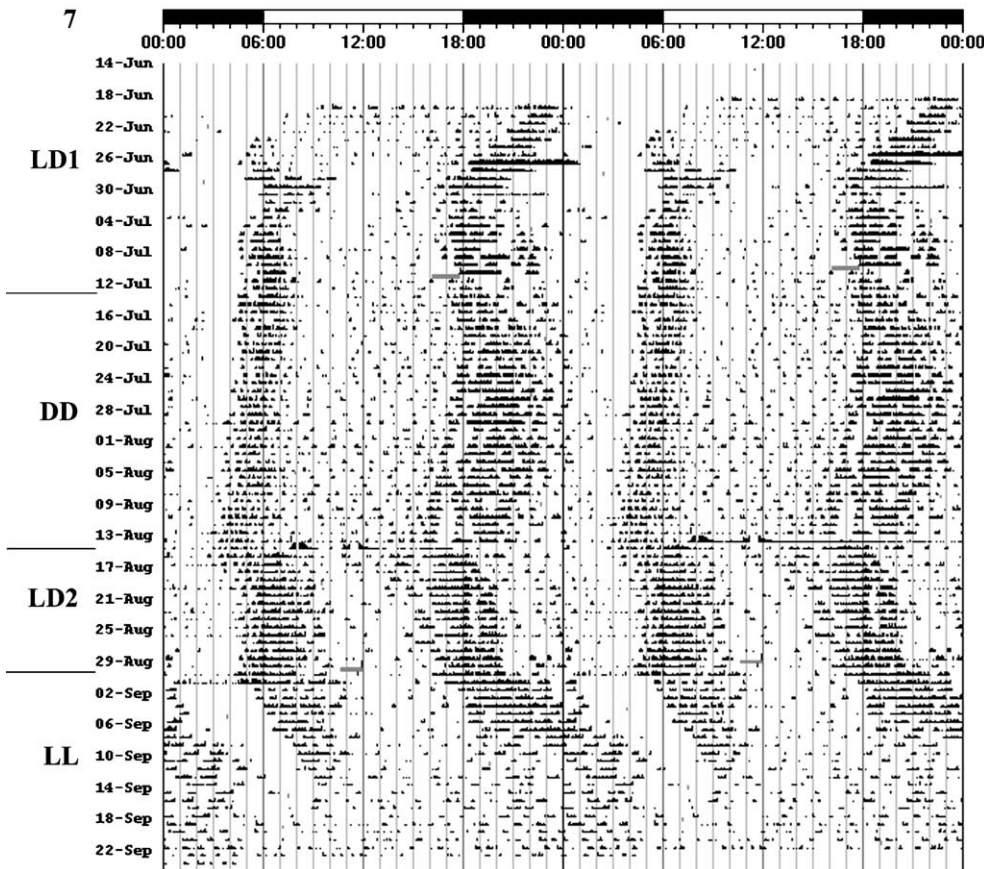


Fig. 3. The locomotory actogram of mouse no. 7 revealed more activity performed during the dark phase and subjective night than during the light phase and subjective day of a 12:12 LD cycle and DD, respectively.

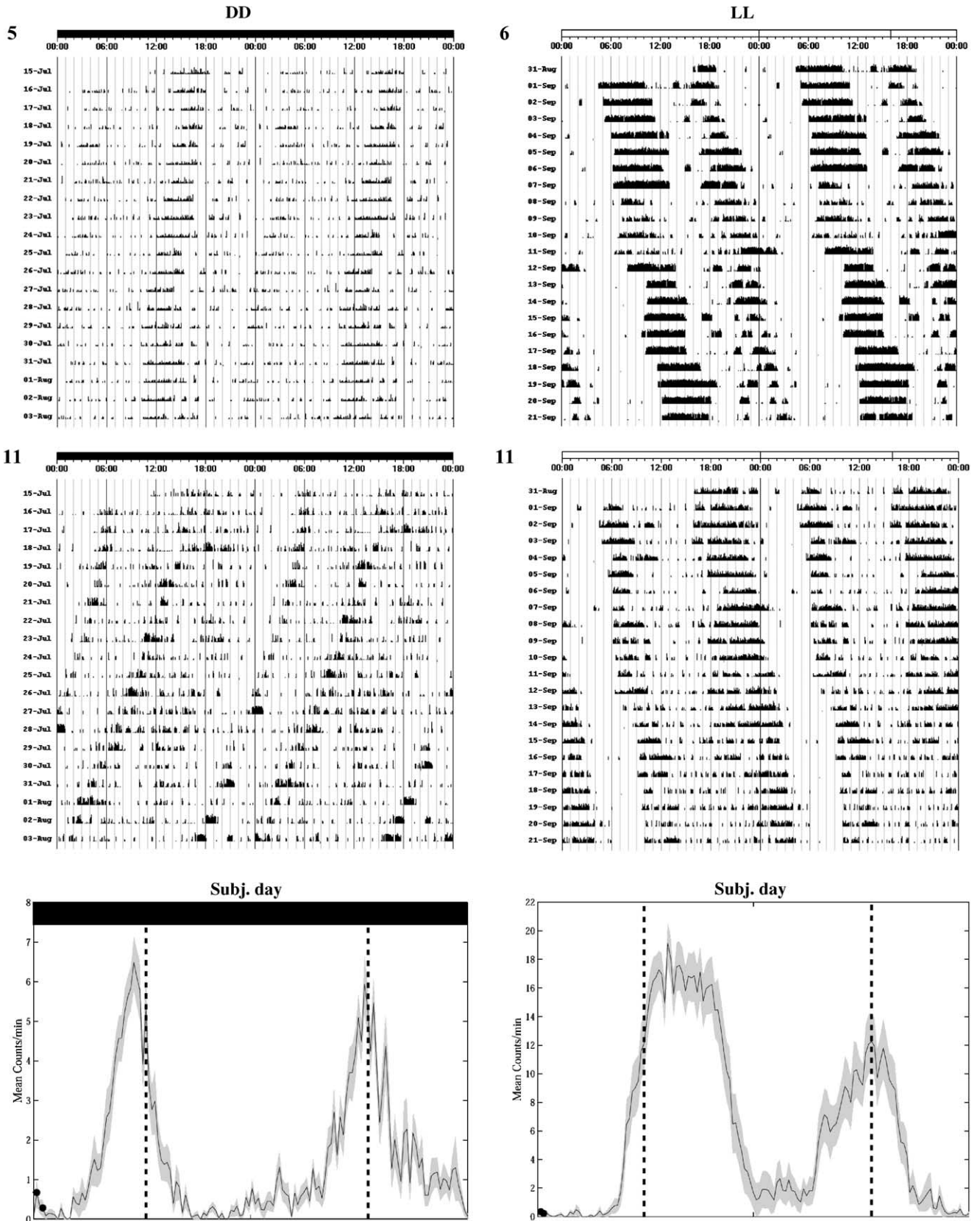


Fig. 4. All *R. pumilio* exhibited free-running of the endogenous rhythms during constant conditions of DD (animal nos. 5 and 11) and LL (animal nos. 6 and 11). Activity profiles, calculated using Clocklab, for animal no. 2 and animal no. 6 during DD and LL, respectively. The rest period during the middle of the day is maintained.

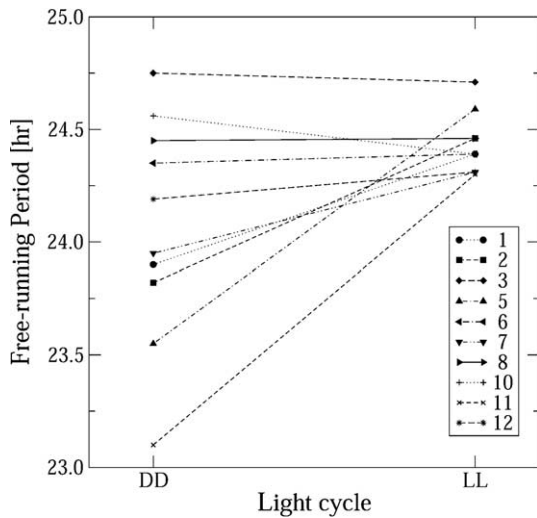


Fig. 5. Free-running periodicity of the endogenous rhythms ( $\tau$ ) during constant dark (DD) and constant light (LL). In LL,  $\tau > 24$  h and there is less inter-individual variation than that observed in DD.

including dawn and dusk transitions since light varies continuously during twilight periods. Using only the animals initially exposed to twilight conditions in LD1, their average phase-angle difference between activity onset and lights on for LD2 and LD3 was calculated. On average, the onset of activity occurring in LD2–3 was 48 min before lights on. The average time of activity onset for the animals in the natural conditions was  $5:42 \pm 0:12$  h. Thus, the average time of the beginning of the ‘day period’ corresponded to 6:30 h, which is close to civil twilight. Using the same method of calculation for the phase-angle difference for activity offset ( $20:23 \pm 0:31$  h) in relation to lights off (50 min), the end of the ‘day period’ occurred at 19:33 h. Light levels at 6:30 h were 4.7 lx (0.44 ft candles) and, at the end of the day, 0.0044 lx (0.00041 ft candles).

There is no difference between the lengths of the average activity period of animals in a square light cycle (average =  $14.10 \pm 0.33$ ) and those in a twilight light cycle (average =  $14.68 \pm 0.67$  h). Qualitatively, there appeared to be no difference between the animals on a square light cycle and those on a twilight cycle.

#### 4. Discussion

In rodents, rhythms of running wheel activity are measured as an overt output of the circadian clock [22]. Although the site of differentiation between nocturnality and diurnality is unknown, animals are characterised as being nocturnal, diurnal or crepuscular based on the percentage of their activity that occurs during the night, day or twilight periods, respectively. This is the first study to determine light–dark entrainment in *R. pumilio*. *R. pumilio* shows stable entrainment, which suggests that the light–dark cycle is a potent zeitgeber for this animal as in most other mammals [23]. Eighty-three percent of four-striped field

mice were diurnal under all lighting cycles including during constant conditions, where *R. pumilio* showed a higher percentage of activity during the subjective day than during the subjective night. The calculation of percentage diurnality during constant conditions had not been performed before, but it could be a good indicator of the animal’s activity pattern because it is a reflection of the endogenous clock.

The average percentage diurnality of *R. pumilio* was 68% in a light–dark cycle this is comparable to the Indian palm squirrel, *Funambulus pennanti*, which has an average percentage diurnality of 88% [23], and *A. niloticus*, which has an average percentage diurnality of 60% [24]. The similarity in diurnality between *R. pumilio* and *A. niloticus* may be attributed to the fact that both species have a bimodal activity rhythm with a bout of activity in the morning and in the evening with a rest period in the middle of the day. The Indian palm squirrel, on the other hand, has a continuous activity rhythm during the day. As with *O. degus*, the rest period is attributed to the animal’s intolerance of high temperatures, but, since the same behaviour was apparent in the laboratory and under constant conditions, it is more feasible that the bimodality is an intrinsic feature [25].

There were inter-individual differences in  $\alpha$  between the three light–dark cycles. There was no pattern in the changes but the variations in  $\alpha$  after DD could be related to the variations in  $\tau$  during DD.  $\alpha$  changed as the light cycles changed, with a significant difference in  $\alpha$  between LD2, DD and LL, which corresponded to observations made on *Arvicanthis* [24]. This agreed with the predictions made by [19] that  $\alpha$  increases with increasing light intensity.

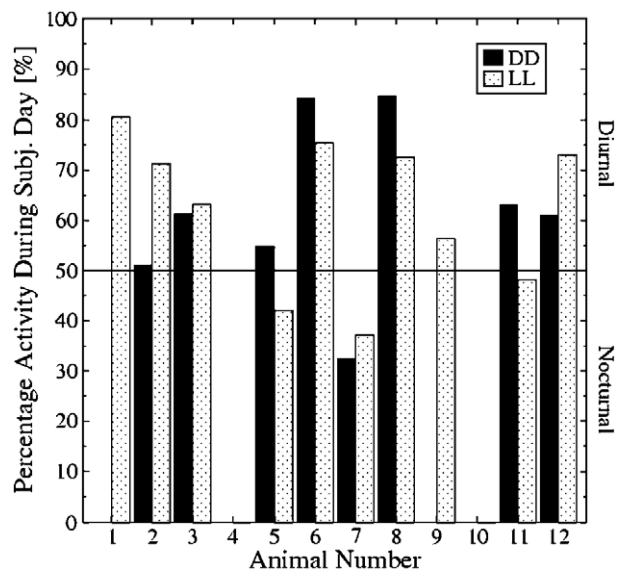


Fig. 6. Percentage activity during subjective day was calculated as a percentage of the total activity. Only one animal showed activity lower than 50% during subjective day for both DD and LL. Of the 8 animals that have complete data sets, two animals showed contradictory behaviour, being nocturnal in one condition and diurnal in another.

Generally,  $\tau$  in constant dark ( $\tau_{DD}$ ) differs from  $\tau$  in constant light ( $\tau_{LL}$ ) and Aschoff's rule hypothesizes that these changes are opposite in diurnal and nocturnal animals [19].  $\tau$  during constant light is less than  $\tau$  during constant dark in diurnal animals and vice versa in nocturnal animals. This rule was based on a limited amount of information and was refined by Daan and Pittendrigh [18]. The new theory states that  $\tau$ , the free-running period in constant light lengthens in species with  $\tau_{DD} < 24$  h and shortens in species with  $\tau_{DD} > 24$  h, irrespective of whether the animal is diurnal or nocturnal.

All *R. pumilio* showed distinct free-running periods. There were large inter-individual variations in  $\tau$  during DD, which agrees with results found by [26] for diurnal mammals. The average  $\tau_{DD}$  for *R. pumilio* was 24:03 h and for  $\tau_{LL}$  24:26 h. Although average  $\tau_{DD}$  was close to 24 h, the variability in  $\tau$  was large and this tends to disagree with the findings of Pittendrigh and Daan [27] who state that the variability in  $\tau$  is smaller in species with an average  $\tau$  closer to 24 h. In *R. pumilio*,  $\tau_{LL}$  increased when  $\tau_{DD} < 24$  h, which disagrees with Aschoff's rule but conforms well to the prediction that if  $\tau_{DD} < 24$  h then it will lengthen in constant light, regardless of whether the animal is diurnal or nocturnal, as is suggested by [18]. The prediction further states that if  $\tau_{DD} > 24$  h it shortens in constant light and can possibly be refined, since in *R. pumilio* when  $\tau_{DD} > 24$  h it either remained the same or decreased slightly. The fact that  $\tau$  increased or decreased in LL was not an after-effect of the transient motion that brought the animals into this steady state. Transients are cycles of rapidly changing duration intervening between two steady states [27]. Experiments by Pittendrigh and Daan [27] showed that hamsters who phase-delay when placed in a 12:12 LD cycle from a DD cycle also show an after-effect of the sign of the phase shifts in the second free-run, i.e. the animal would show a decrease in  $\tau$  in the second free-run. This was not apparent for *R. pumilio*. There is less variation in  $\tau_{LL}$  than in  $\tau_{DD}$ . Although splitting did not occur during LL, the fact that the striped field mouse had a bimodal activity pattern provides evidence for the presence of a dual oscillator.

The M oscillator was strongly linked to dawn, as the onsets of activity were fairly constant, whereas the E oscillator was not as strongly linked to dusk, since the offsets were imprecise. More recently, the M and E oscillators have been linked to specific genes. It is conceptualised that season is gauged at the molecular level in the SCN by the expression of circadian genes (*per1*, *cry1*, *per2* and *cry2*) [28]. *Per1/cry1* oscillator is accelerated by light and decelerates in the dark hence tracking dawn. The *per2/cry2* oscillator is decelerated by light and accelerated by dark thus tracking dusk. As a consequence, we have a perfect molecular mechanism for measuring season [28].

During the first light–dark cycle, six animals were placed in natural conditions and six were placed in artificial conditions. Since there are three distinct definitions of twilight, it was difficult to determine the exact time 'lights

on' was perceived since the animals were not equipped with light-sensitive radio collar transmitters as used by [20] on the European ground squirrel. Three definitions of twilight are recognized based on the position of the sun, astronomical, nautical and civil. Astronomical twilight is defined as the period when the centre of the sun is 18° below the horizon. Nautical twilight occurs when the centre of the sun is 12° below the horizon and civil twilight occurs during the interval between sunset and the time when the sun is 6° below the horizon. Civil twilight is used to calculate circadian parameters when the animals are in a twilight cycle because the largest transition between light and dark occurs during this period [20,29], but comparing the percentage diurnality for the three twilights showed different activity patterns in two of the five *R. pumilio*. Pittendrigh and Daan [30] stated that the choice of twilight is arbitrary since there is no way of knowing when an animal 'perceives' light, at the beginning of day. Unlike humans or any rodents, *R. pumilio* has 50% cones and 50% rods (A. Lukats, personal communication). Cones are adapted for photopic vision and rods are adapted for scotopic vision. Since there is an abundance of both photoreceptors, these mice should be able to see well in both dim and bright light. According to Ahnelt [10], the European ground squirrel, which does not use dawn or dusk for entrainment, has 5% rods in relation to cones. Experiments performed under twilight conditions are important since twilight has been found to widen the range of entrainment observed in LD twilight. Furthermore, twilight transitions increase the strength of the LD zeitgeber in hamsters [31]. No significant difference was found in the period during which the animals were active ( $\alpha$ ) for striped field mice on either square LD cycle or those on a twilight cycle. This finding is contradictory to that found by Boulos et al. and Tang et al. [32,33]. The striped field mice exposed to twilight conditions may have been influenced by non-photoc environmental stimuli such as temperature, humidity and differing light intensity levels throughout the day that could not be controlled for. During June–July, the humidity in the highveld of South Africa is very low. It is unlikely that activity onset and offset were triggered by temperature changes since *Rhabdomys* only appear to avoid activity in the heat of the day. It is more likely that the light–dark transition is the principal entraining agent in *Rhabdomys* as is similarly shown in the cohort of mice that were exposed to the square wave lighting regime where temperature and relative humidity were controlled for. There was a significant difference between the time of onset and offset of animals on a square light–dark cycle compared to those on a twilight cycle. Animals on a square light–dark cycle started their activity earlier and ended their activity earlier than those animals on a twilight cycle. Similarly, in squirrel monkeys and hamsters, the activity onset occurred later and activity offsets earlier during the square light cycle compared to the twilight cycle [32,33]. It is of fundamental interest that, under civil twilight, the four-striped field mouse exhibits a marked



diurnality in its locomotory activity pattern. The strict diurnality of the animal and the marked response in its behavioural activity under twilight conditions could prove important for future research into unravelling the nature of the M and E oscillators at the molecular level by investigating the expression of *per1* and *per2* and *cry1* and *cry2* circadian gene expression in the SCN.

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## References

- [1] Sato T, Kawamura H. Effects of bilateral SCN lesions on the circadian rhythms in a diurnal rodent, the Siberian chipmunk (*Eutamias sibiricus*). *J Comp Physiol A* 1984;155:745–52.
- [2] Nunez AA, Bult A, McElhinny TL, Smale L. Daily rhythms of Fos expression in hypothalamic targets of the suprachiasmatic nucleus in diurnal and nocturnal rodents. *J Biol Rhythms* 1999;14(4):300–6.
- [3] Sato T, Kawamura H. Circadian rhythms in multiple unit activity inside and outside the suprachiasmatic nucleus in the diurnal chipmunk (*Eutamias sibiricus*). *Neurosci Res* 1984;1:45–52.
- [4] Zlomanczuk P, Margraf RR, Lynch GR. In vitro electrical activity in the suprachiasmatic nucleus following splitting and masking of wheel-running behavior. *Brain Res* 1991;559:94–9.
- [5] Smale L, Castleberry C, Nunez AA. Fos rhythms in the hypothalamus of *Rattus* and *Arvicanthis* that exhibit nocturnal and diurnal patterns of rhythmicity. *Brain Res* 2001;899:101–5.
- [6] Ahnelt PK. Characterization of the color related receptor mosaic in the ground squirrel retina. *Vision Res* 1985;25(11):1557–67.
- [7] Kryger Z, Galli-Resta L, Jacobs GH, Reese BE. The topography of rod and cone photoreceptors in the retina of the ground squirrel. *Vis Neurosci* 1998;15(4):685–91.
- [8] Hut RA, van Oort BEH, Daan S. Natural entrainment without dawn and dusk: the case of the European ground squirrel (*Spermophilus citellus*). *J Biol Rhythms* 1999;14(4):290–9.
- [9] Calderone JB, Jacobs GH. Cone receptor variations and their functional consequences in two species of hamster. *Vis Neurosci* 1999;16(1):53–63.
- [10] Lee TM, Labyak SE. Free-running rhythms and light- and dark-pulse phase response curves for diurnal *Octodon degus* (Rodentia). *Am J Physiol* 1997;273:R278–86.
- [11] Kas MJ, Edgar DM. A nonphotic stimulus inverts the diurnal–nocturnal phase preference in *Octodon degus*. *J Neurosci* 1999;19(1):328–33.
- [12] Blanchong JA, McElhinny TL, Mahoney MM, Smale L. Nocturnal and diurnal rhythms in the unstriped Nile rat. *J Biol Rhythms* 1999;14(5):364–77.
- [13] Zisapel N, Barnea E, Izhaki I, Anis Y, Haim A. Daily scheduling of the golden spiny mouse under photoperiodic and social cues. *J Exp Zool* 1999;284:100–6.
- [14] Brooks PM. The ecology of the four-striped field mouse, *Rhabdomys pumilio* (Sparman, 1784), with particular reference to a population on the van Riebeeck Nature Reserve, Pretoria. PhD thesis, Faculty of Science, University of Pretoria. 1974.
- [15] Johnson AG. The social organisation and behaviour of the striped field-mouse *Rhabdomys pumilio* (Sparman 1784): studies in captivity and in the field. Master's thesis, Faculty of Science, University of Cape Town. 1980.
- [16] David JHM. Demography and population dynamics of the striped field mouse *Rhabdomys pumilio*, in alien acacia vegetation on the Cape Flats, Cape Province, South Africa. PhD thesis, University of Cape Town. 1980.
- [17] Marais FJ. The behaviour and population dynamics of a confined colony of striped mice (*Rhabdomys pumilio*). Master's thesis, Faculty of Science, University of Pretoria. 1974.
- [18] Daan S, Pittendrigh CS. A functional analysis of circadian pacemakers in nocturnal rodents: III. Heavy water and constant light: homeostasis of frequency? *J Comp Physiol A* 1976;106:267–90.
- [19] Aschoff J. Circadian rhythms: influences of internal and external factors on the period measured in constant conditions. *Z Tierpsychol* 1979;49:225–49.
- [20] Hut RA, Mrosovsky N, Daan S. Nonphotic entrainment in a diurnal mammal, the European ground squirrel (*Spermophilus citellus*). *J Biol Rhythms* 1999;14(5):409–19.
- [21] Pittendrigh CS. Circadian rhythms and the circadian organization of living systems. *Cold Spring Harb Symp Quant Biol* 1960;25:159–82.
- [22] Kornhauser JM, Ginty DD, Greenberg ME, Mayo KE, Takahashi JS. Light entrainment and activation of signal transduction pathways in the SCN. *Prog Brain Res* 1996;111:133–47.
- [23] Rajaratnam SMW, Redman JR. Light–dark entrainment of circadian activity rhythms of the diurnal Indian palm squirrel (*Funambulus pennanti*). *Biol Rhythm Res* 1999;30(4):445–66.
- [24] Katona C, Smale L. Wheel-running rhythms in *Arvicanthis niloticus*. *Physiol Behav* 1997;61(3):365–72.
- [25] Garcia-Allegue R, Lax P, Madariaga AM, Madrid JA. Locomotor and feeding activity rhythms in a light-entrained diurnal rodent, *Octodon degus*. *Am J Physiol* 1999;277:R523–31.
- [26] Pohl H. Characteristics and variability in entrainment of circadian rhythms to light in diurnal rodents. In: Aschoff J, Daan S, Goos FA, editors. Vertebrate circadian systems: structure and physiology. Berlin: Springer-Verlag; 1982.
- [27] Pittendrigh CS, Daan S. A functional analysis of circadian pacemaker in nocturnal rodents: I. The stability and lability of spontaneous frequency. *J Comp Physiol A* 1976;106:223–52.
- [28] Daan S, Albrecht U, van der Horst GTJ, Illnerova H, Roenneberg T, Wehr TA, et al. Assembling a clock for all seasons: are there M and E oscillators in the genes? *J Biol Rhythms* 2001;16:105–16.
- [29] Hut RA. Natural entrainment of circadian systems: a study in the diurnal ground squirrel *Spermophilus citellus*. PhD thesis, Rijksuniversiteit Groningen. 2001.
- [30] Pittendrigh CS, Daan S. A functional analysis of circadian pacemaker in nocturnal rodents: IV. Entrainment: pacemaker as clock. *J Comp Physiol A* 1976;106:291–331.
- [31] Boulos Z, Macchi MM, Terman M. Twilights widen the range of photic entrainment in hamsters. *J Biol Rhythms* 2002;17:353–63.
- [32] Boulos Z, Macchi M, Terman M. Effects of twilights on circadian entrainment patterns and reentrainment rates in squirrel monkeys. *J Comp Physiol A* 1996;179:687–94.
- [33] Tang I-H, Murakami DM, Fuller CA. Effects of square-wave and simulated natural light–dark cycles on hamster circadian rhythms. *Am J Physiol* 1999;276:R1195–202.