Locomotor activity in the Namaqua rock mouse (*Micaelamys namaquensis*) – Entrainment by light manipulations

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Abstract

The locomotor activity rhythms of wild-caught Namaqua rock mice (*Micaelamys namaquensis* Smith, 1834) were examined under four light cycle regimes in order to quantitatively describe the daily expression of locomotor activity and to study the innate relationship between activity and the light-dark cycle. Activity was always significantly higher at night than in the day: 1) The LD1 light cycle (12L: 12D) established a distinct light-entrained and strongly nocturnal activity rhythm (99.11% nocturnal activity). The activity onset was prompt (ZT 12.2 ± 0.04) and activity continued without any prominent peaks or extended times of rest until the offset of activity at ZT 23.73 ± 0.08. 2). Evidence for the internal maintenance of locomotor activity was obtained from the constant dark cycle (DD) in which locomotor activity free ran (mean τ = 23.89 h) and 77.58% of the activity was expressed during the subjective night. 3) During re-entrainment (LD2; 12L: 12D), a nocturnal activity rhythm was re-established (98.65% nocturnal activity) and, 4) the inversion of the light cycle (DL; 12D: 12L) evoked a shift in activity that again revealed dark-induced locomotor activity (95.69% nocturnal activity). Females were consistently more active than males in all of the light cycles, but only under the DD and LD2 cycles were females significantly more active than males. Although this species is considered nocturnal from field observations, information regarding its daily expression of activity and the role of light in its entrainment is lacking. To the best of our knowledge this study is the first to report quantitatively on the species' daily rhythm of activity and to investigate its relationship to the light-dark cycle.

Key words: Circadian rhythm, entrainment, free-run, locomotor activity, *Micaelamys namaquensis*, Namaqua rock mouse

Introduction

The perpetual rotation of the earth on its axis and the revolution of the earth around the sun are undoubtedly some of the strongest cyclical events to which organisms are exposed. As a consequence, the light-dark cycle reliably exposes daily and seasonal time and is considered the primary *zeitgeber* (environmental cue) that entrains daily physiological and behavioural rhythms (Benstaali et al. 2001; Reppert and Weaver 2002). Since the availability of environmental resources change in concurrence with changing levels of illumination across the day, the temporal organization of the locomotor activity rhythm of a species is vital. During the process whereby the temporal niche of a species is shaped, the locomotor activity rhythm may become entrained to other non-photic cues and thereby mask the underlying photically-entrained rhythm (Edmonds and Adler 1977; Francis and Coleman 1988; Hut et al. 1999; Rajaratnam and Redman 1999). Consequently, light-entrained daily rhythms that persist (free-run) in an environment devoid of *zeitgebers* confirm the existence of an internal biological clock mechanism which in turn controls the circadian rhythm (Aschoff 1981). In mammals, the suprachiasmatic nucleus (SCN) is considered the central moderator of the circadian timing system (Moore and Eichler 1972; Stephan and Zucker 1972). The importance of the circadian timing system and its coordination by the light-dark cycle is best demonstrated by the prevalence of increased health risks and in some cases disturbances within ecological systems, that result from

disruptions of the circadian clock network and distrainment by a natural light-dark cycle (Bird et al. 2004; Navara and Nelson 2007; Haim et al. 2010; Rotics et al. 2011; Haim and Portnov 2013).

Mammalian daily locomotor activity rhythms have been extensively used in studying photic entrainment of the circadian timing system and in unraveling its underlying mechanisms (Aschoff, 1981; Benstaali et al., 2001). A small number of laboratory-reared mammalian species (e.g. strains of mice, rats and hamster) are typically used in chronobiological studies, as they breed readily and are maintained easily in captivity. Moreover, studies on wild animals usually focus on species from temperate regions of the Northern hemisphere. Here, we study light entrainment of the locomotor activity rhythm in a southern African rodent namely the Namaqua rock mouse (*Micaelamys namaquensis* Smith, 1834). The species is polygynous, shows no sexual dimorphism and inhabits a range of biomes but usually prefers rocky environments above other habitat types (Skinner and Chimimba 2005; Russo et al. 2010). The Namaqua rock mouse is described as communal or social, though such groups may potentially only consist of family members (Fleming and Nicolson 2004). The reproductive system of the Namaqua rock mouse, as well as that of the closely related Tete veld rat (*Aethomys ineptus*), has been shown to be photoresponsive and the species is classified as a seasonal breeder (Muteka et al. 2006). Although various aspects of the Namaqua rock mouse are well-known, basic characteristics of its temporal locomotor activity rhythm have not been studied (Skinner and Chimimba 2005). The species is considered nocturnal mostly based on field observations, but it is possible that the innate circadian rhythm of this species is

not expressed in its natural environment due to masking by other non-photic factors (Mrosovsky 1999). Lovegrove and Heldmaier (1994) have described the species' daily body temperature rhythm and briefly reported on its daily locomotor activity rhythm under a long day photoperiod (16L: 8D) and a high ambient temperature (30°C). Both rhythms were described as displaying a bimodal nocturnal pattern.

The present study aimed to quantitatively describe the daily expression of locomotor activity in male and female Namaqua rock mice and to study the relationship between activity and the level of illumination using various lighting regimes, under controlled laboratory conditions. The mice were also subjected to constant darkness in order to describe the endogenous locomotor activity rhythm of the Namaqua rock mouse and thus to calculate the period of the free running rhythm, which we predicted to be less than 24 h as it is for most nocturnal species (Aschoff 1981). Furthermore, the objective was to re-entrain the mice to a regular light-dark cycle and subsequently expose them to an abrupt inversion of the light-dark cycle as a means to further validate light entrainment of the activity and to further establish whether darkness induces activity.

2. Materials and methods

2.1 Animal housing

The project was approved by the Animal Ethics Committee of the University of Pretoria, Pretoria, South Africa (EC063-11). In mid-August, eight adult *M. namaquensis* (four males; four females; mean body mass = 36.3 ± 2.66 g) were collected from amongst the rocky hills of the Goro Game Reserve in the Soutpansberg region (Limpopo Province,

South Africa). The animals were kept individually in semi-transparent plastic cages (58 x 38 x 36 cm) in a light and temperature controlled animal room at an ambient temperature of 25 °C (± 1°C) and approximately 60% relative humidity. Each cage contained a layer of wood shavings (± 3 cm thick) for bedding and the animals were given a small plastic shelter and tissue paper for nesting material. The mice had *ad libitum* access to food and water and were fed at random times every second or third day to avoid activity entrainment to the feeding schedule. Water and food (parrot seed mix) were topped up and fresh food was replaced. The room was illuminated at day time by white fluorescent lights (± 400 lux) and two weeks prior to the experimentation period, the animals were maintained on a 12L: 12D light-dark cycle (L: 06:00 – 18:00h).

2.2 Activity recording and experimental procedure

Activity was recorded using infra-red motion captors (Quest PIR internal passive infrared detector; Elite Security Products (ESP), Electronic Lines, London, UK) that were attached to the top of each cage and detected movement over the whole cage floor area. The number of locomotory movements detected during each minute was stored on a computer using VitalView software (VitalView^{TM}, Minimitter Co., Sunriver, OR, USA; http://www.minimitter.com). Methods were similar to those employed in Van der Merwe et al. 2011. Four consecutive light cycle regimes, each lasting for fourteen days (the second light cycle lasted 15 days), were used to measure locomotor activity responses: 1) The LD1 light cycle comprised of 12L: 12D (ZT 0 at 06:00 and ZT 12 at 18:00h) for recording any patterns of activity entrainment to the L/D cycles and in order to ensure that animals are

entrained to a known light cycle to obtain a starting point for determination of endogenous rhythms. 2) Animals were exposed to constant darkness (DD) to assess whether animals possess endogenous rhythms of locomotor activity and if it is the case, to determine the period of the free running rhythm (tau; τ). 3) A second light cycle comprising of 12L: 12D (ZT 0 at 06:00 and ZT 12 at 18:00h) served to re-entrain the animals to a 12L:12D light cycle before exposing them to the last light cycle regime. 4) An inverse light cycle (DL; ZT 0 at 18:00 and ZT 12 at 06:00h), in order to subject the animals to a drastic change in the L/D-cycles and evaluate the response observed in locomotor activity. Inversing the light cycle will establish whether animals use light to entrain their activity rhythms as opposed to some other cue, and in addition provide an indication of the rate of re-entrainment to a novel light cycle. During the transition from the LD2 light cycle to the inverse DL light cycle, the animals were exposed to a 24 h period of light, which was then followed by D12: L12. Note: Zeitgeber time (ZT) refers to time in hours during the LD or DL light cycles (ZT 0 = light phase onset; ZT 12 = dark phase onset).

2.3 Data analysis

The recorded activity data were analyzed and the daily activity rhythms visually presented as double-plotted actograms using the computer program ActiView (ActiView^{TM}, Minimitter Co., Sunriver, OR, USA; http://www.minimitter.com). Only the last 10 days of each light cycle were used in the calculations as to exclude the transitory periods between light cycles. Moreover, under the LD2 and DL light cycle, only the days in which the animals were fully re-entrained were included in the analyses. Activity counts and

percentages of activity were compared between the four light cycles as well as within each light cycle (light phase vs. dark phase) for all of the mice combined as well as for males and females separately. The sums of the activity counts per light phase and per dark phase of each day were calculated for each individual across all four light cycles. These values were then used to estimate the mean number of activity counts (of all individuals combined) for each entire light cycle as well as for the light phase and dark phase of each light cycle separately. The number of activity counts during either the light or dark phase (or subjective light or dark phase under DD) of each day was further expressed as a percentage against the total number of activity counts for each day and was calculated for all animals individually. The mean of these values within each of the light cycle regimes were then presented as the percentage of activity. The period length (in hours) during the DD cycle was calculated using the chi-squared periodogram function in the rhythm analysis software Clocklab (ClockLab TM, Actimetrics, Evanston, IL, USA). The activity percentages for the subjective light and dark phases were calculated for each individual according to its own τ. Where it was possible, we determined the mean daily onset and offset times for each animal. Activity onset was defined visually as activity within ten consecutive minutes and activity offset as inactivity for 20 minutes. From this we extrapolated the phase angle of entrainment (ψ; the time difference between the lights on or off and activity onset or offset) and the active phase (α; the period from activity onset to activity offset).

Statistical analyses were performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and IBM SPSS Statistics version 21.0 (SPSS Inc., Chicago, IL,

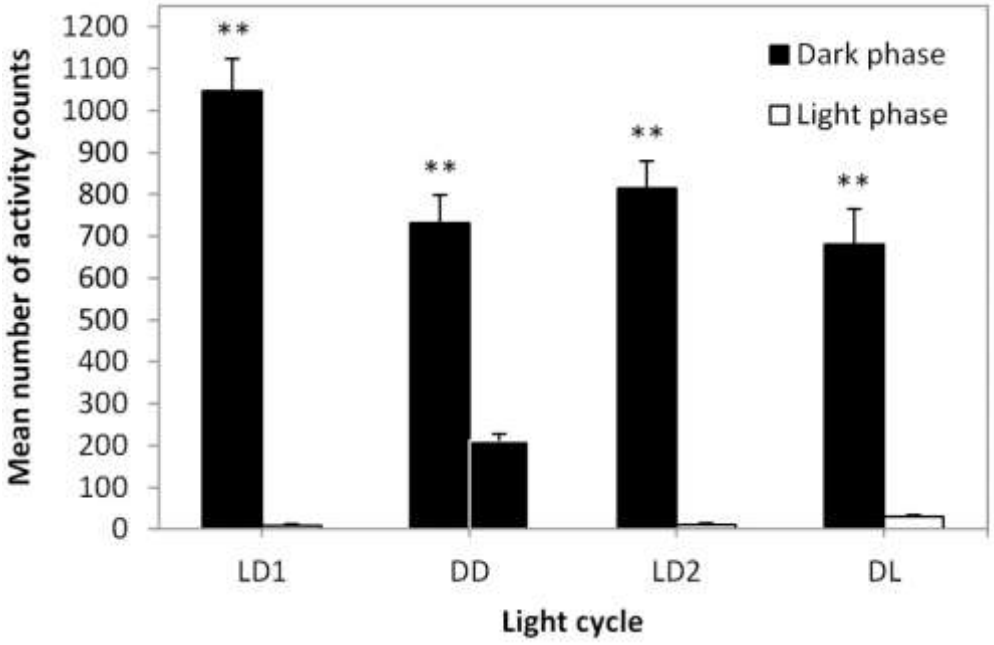
USA). Statistical significance for light cycle (LD1, DD, LD2 and DL), light phase (light/dark phase and subjective light/dark phase), sex, and the interaction effects of light cycle with light phase as well as light cycle with sex on activity were determined. Data were not normally distributed and thus a generalized linear mixed model was used to evaluate activity; the *post hoc* least significant difference test was used where significant differences were detected. $P<0.05$ were considered significantly different and all means were indicated with standard error (SE). To obtain means for activity counts during the light and dark phases, summed hourly values for the dark phases and the light phases were divided by the 12 hours of the respective phases, whereas the total daily activity values (summed hourly values) were divided by the 24 hours of the entire day.

3. Results

3.1 The LD1 light cycle

The Namaqua rock mouse displayed a robust daily locomotor activity rhythm in accordance with the alternation of light and dark, and was invariably nocturnal across all light cycles (Figs. 1, 2 and 3). All of the animals were nearly exclusively active during the dark phase of the LD1 light cycle (99.11% nocturnal activity); the mean amount of activity counts were significantly higher during the dark phase (mean for 12h dark phase: 1047.87 ± 76.57) than during the light phase (mean for 12h light phase: 9.37 ± 2.88; $F=184.161$, $df_1=1$, $df_2=487$, $P<0.001$; Fig. 1). The mean amount of activity counts for the 24h day for the whole LD1 cycle was 528.62 ± 38.37 with no significant effect of sex on the activity counts (males: 537.34 ± 54.99, females: 519.91 ± 53.51, $F=0.052$, $df_1=1$, $df_2=487$,

Figure 1 : Mean numbers of activity counts for the Namaqua rock mouse (*Micaelamys namaquensis*) showing significantly higher (P<0.001) values in the 12 hours of the dark phases (or approximately 12 hours for the subjective dark phase of DD) than in the light phases (or subjective light phase of DD) of all four light cycles. Summed activity counts for the 12 hours light /dark were divided by 12 to obtain a mean. **=P<0.001.



P=0.820; Fig. 4). The mean activity onset during LD1 was precise (ZT 12.2 ± 0.04) and coincided closely with the start of the dark phase. The mean activity offset (ZT 23.73 ± 0.08) occurred prior to the end of the dark phase and the mean ψ and α for LD1 was 0.2 ± 0.04 h and 11.53 ± 0.11 h, respectively. Individual differences in the activity rhythms, as presented by the actograms were minor; generally activity continued throughout the dark phase without any pronounced peaks or troughs (See Fig. 2). Only in one female mouse (animal no. 8) was activity expressed more intermittently during the latter half of the dark phase.

Figure 2 : An actogram of the locomotor activity rhythm of animal no. 7 across all four light cycles revealing a strong nocturnal activity rhythm during entrainment to a 12L: 12D light cycle (LD1), a free running activity rhythm (τ = 23.7 h) during constant darkness (DD), slow re-entrainment to the dark phase during a second 12L: 12D light cycle (LD2), and finally a shift in activity following an inverse of the light cycle (DL).

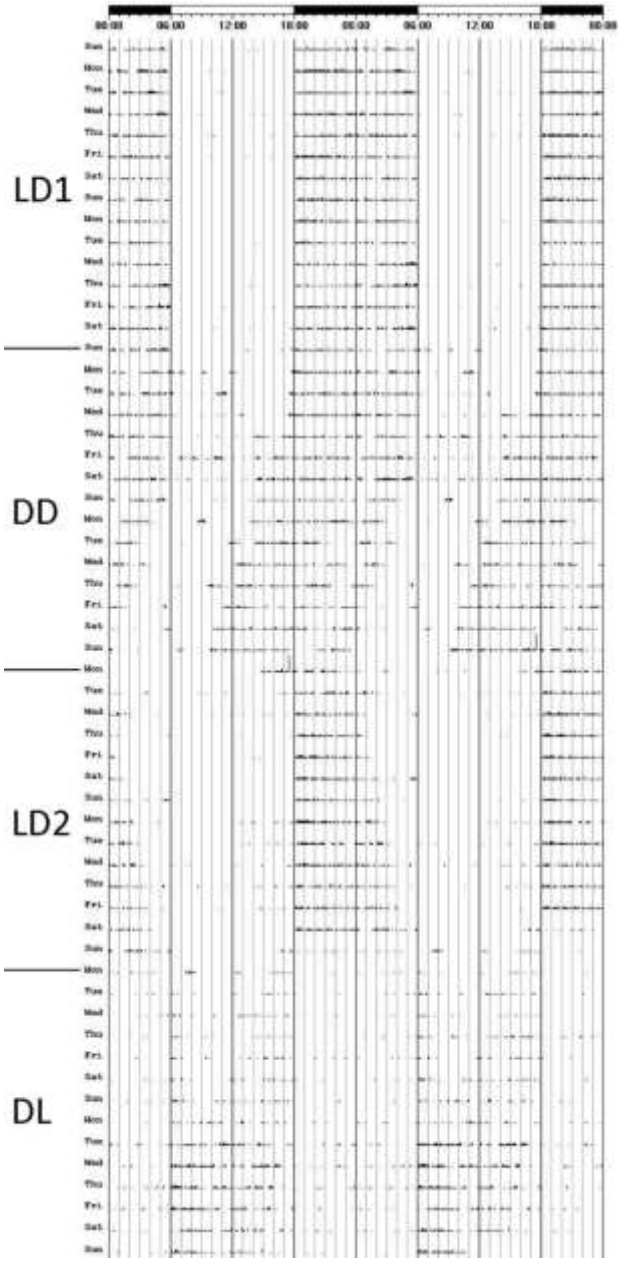
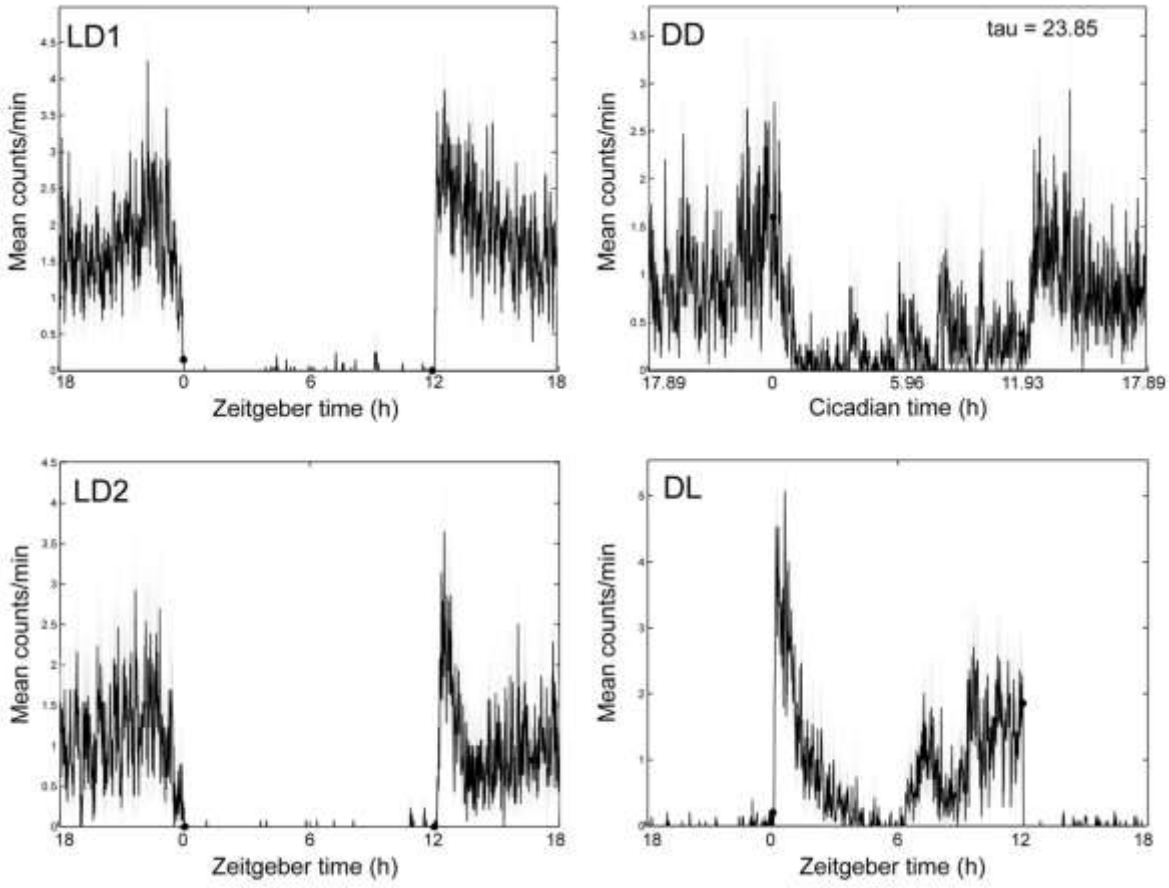


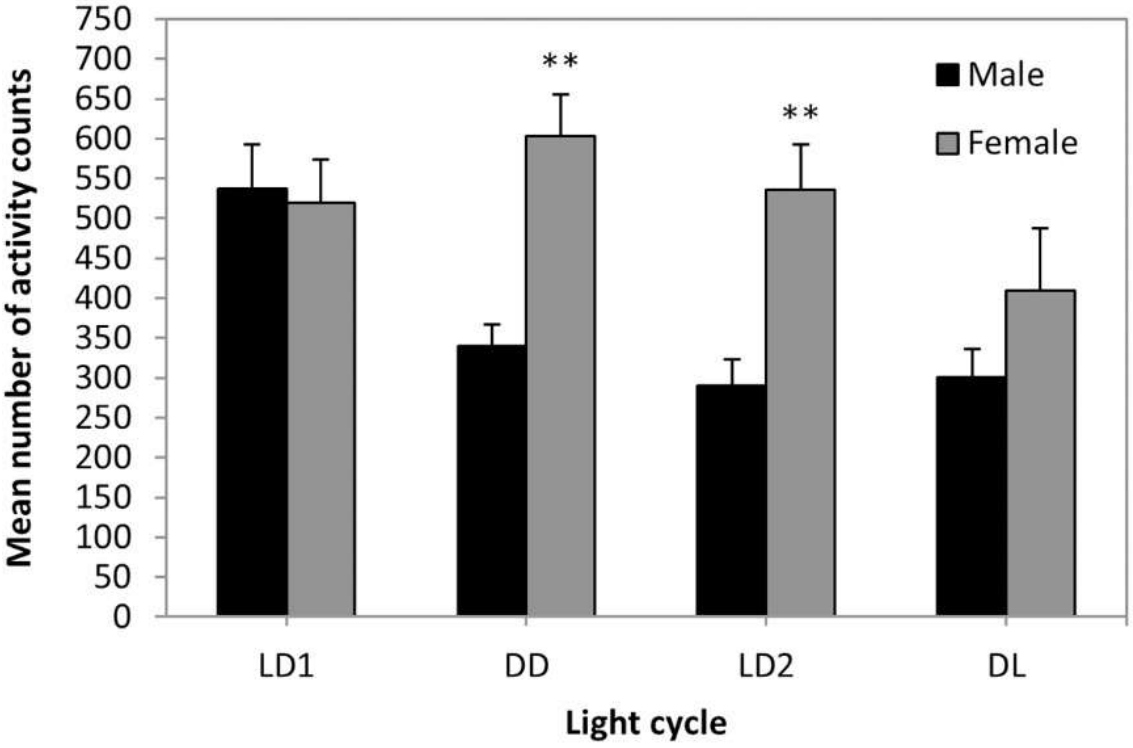
Figure 3: Average activity profiles for animal no. 1 (male) for the last ten days of each of the four light cycles (LD1, DD, LD2 and DL). Zeitgeber time (LD1, LD2 and DL) and circadian time (DD) is indicated on the x-axis and mean counts/min on the y-axis.



3.2 The DD light cycle

Under the DD cycle, all of the mice retained a distinct daily activity rhythm, thus validating the presence of an internal time-keeping mechanism. Compared to the LD1 cycle, the mean amount of activity counts during the DD cycle was slightly lower (741.90 ± 29.42) but with the difference being not significant between the two groups (F=3.654, df_1=3, df_2=487, P=0.240). The mice were significantly more active during the subjective night than the subjective day (77.58% nocturnal activity; F=79.030, df_1=1, df_2=487,

Figure 4 : Mean numbers of activity counts over the 24h day for the Namaqua rock mouse (*Micaelamys namaquensis*) under four light cycles (LD1, DD, LD2 and DL) showing values obtained for males and females separately. Females were significantly more active than males during the DD, LD2 and DL light cycles. Summed activity counts for the 24 hours of the day were divided by 24 to obtain a mean. **=*P*<0.001..



P<0.001) with the mean number of activity counts being 732.22 ± 65.56 and 211.57 ± 15.71 for these two light phases respectively (Fig. 1). The mean number of total activity counts over the 24h day was also significantly higher in females (603.76 ± 52.17) than in males (340.04 ± 27.19, F=20.095, df_1=1, df_2=487, P<0.001; Fig. 4). Only animal no. 5 (male) displayed an increasingly arrhythmic activity pattern following DD. The mean free run period or τ was slightly less than 24 h (23.89 ± 0.08 h) and the range was small (23.4 –

24.13 h); only two animals had a τ marginally above 24 h (animal no. 3, female: 24.05 h and animal no. 5, male: 24.13 h).

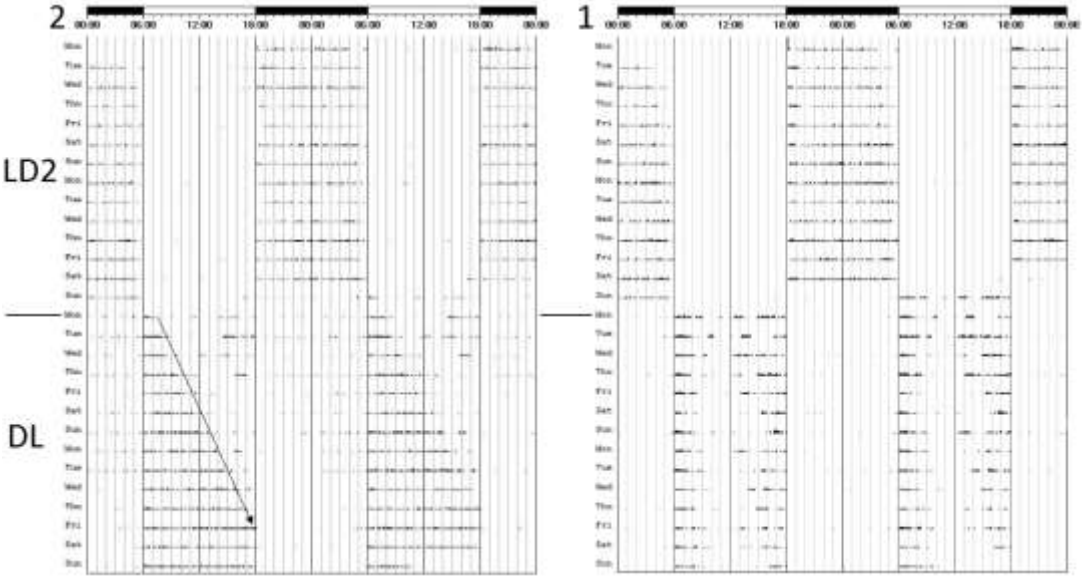
3.3 The LD2 light cycle

During the re-entrainment period (LD2), only mouse no. 7 did not immediately re-entrain to the new light schedule (Fig. 2) and consequently, for this individual, only the last day of its recorded activity was included in the analysis. The overall mean number of activity counts over the 24h day was 413.04 ± 32.80; this value was significantly lower than the value obtained under the LD1 light cycle (F=3.654, df_1=3, df_2=487, P=0.022) yet not significantly different to the value under the DD cycle (F=3.654, df_1=3, df_2=487, P=0.180). The percentage of nocturnal activity was 98.65%, with a significantly higher mean amount of activity counts during the dark phase (814.91 ± 65.41) than the light phase (11.16 ± 2.91; F=151.266, df_1=1, df_2=487, P<0.001; Fig. 1). Under this light cycle, females expressed significantly more activity over the 24h day than males (males: 289.87 ± 33.28, females: 536.20 ± 56.52, F=14.104, df_1=1, df_2=487, P<0.001, Fig. 4). The activity onsets were restored to again coincide close to the start of the dark phase (ZT 12.27 ± 0.04) and the mean ψ was 0.27 ± 0.04 h. Activity offset was on average less precise and occurred slightly earlier than during LD1 (ZT 23.43 ± 0.14) because of inter-individual differences in the rate of re-entrainment to the LD2 light cycle. Subsequently, the mean α was 0.39 h shorter during LD2 (ZT 11.14 ± 0.14 h).

3.4 The DL light cycle

The locomotor activity rhythm of the Namaqua rock mouse was immediately affected by the inverted light-dark cycle (DL). Once more a strong nocturnal rhythm was observed (percentage nocturnal activity: 95.69%) with the mean number of activity counts during the 12h of the dark phase (680.36 ± 85.19) being significantly higher than during the light phase (30.62 ± 4.72; F=58.116, df_1=1, df_2=487, P<0.001; Fig. 1). Over the 24h day, females were more active than males, but the difference was not significant (males: 300.99 ± 35.69, females: 409.99 ± 70.60, F=1.628, df_1=1, df_2=487, P=0.203; Fig. 4). The overall mean number of the activity counts over the 24h day (355.49 ± 42.71) was significantly lower than during the LD1 light cycle (F=3.654, df_1=3, df_2=487, P=*0*.003) as well as the DD light cycle (F=3.654, df_1=3, df_2=487, P=0.025). Activity onset (ZT 12.09 ± 0.02) remained in close proximity to the start of the dark phase (ZT 12 = 06:00) and the mean ψ was 0.09 ± 0.02 h. The actograms revealed a relative amount of inter-individual differences in the activity profiles displayed; re-entrainment times ranged between 0 and 10 days. Only two mice (both males) displayed immediate re-entrained activity rhythms to the DL light cycle. In one of these males, the pattern of activity was less pronounced under DL (Fig. 2.) and in the other male, the mode of activity changed from continuous (during LD2) to bimodal (during DL; Fig. 5) but with the percentage of nocturnal activity being similar between these two light cycles (DL: 98.11 ± 0.33%; LD2: 99.79 ± 0.09%). In the six remaining individuals (four females and two males), re-entrainment was gradual and took approximately 10 days to complete. In two of these mice, only the last five days of the recorded activity data were used in the analyses and in the remaining four mice, only the

Figure 5 : Actograms for the Namaqua rock mouse (*Micaelamys namaquensis*) showing either a progressive shift (on the left; animal no. 2) or a direct shift (on the right; animal no. 1) in the locomotor activity rhythm in response to the inversing of the lighting regime from 12L: 12D (LD1) to 12D: 12L (DL). Note that activity in animal no. 1 changed from continuous during LD2 to bimodal during DL



last three days were used in the analyses. The slowly re-entraining mice generally exhibited a period of continuous activity at the beginning of the night, which then increased daily until the offset of activity coincided with the end of the night (ZT 12 = 18:00h). Simultaneously there was a decrease in the daytime activity and furthermore, shorter bouts of activity were also observed in the latter part of the night (Fig. 5). From three individuals, it was possible to calculate that the continuous active period increased by 0.89 ± 0.5 h each day. Overall, the mice expressed ~3.2% more daytime activity during the

DL cycle than during the LD1 and LD2 cycles, even after the activity offsets were fully restored.

4. Discussion

The locomotor activity rhythms of mammals are typically described in relation to the strongest environmental *zeitgeber*, the light-dark cycle, as being nocturnal, diurnal, crepuscular or cathemeral, but may entrain to *zeitgebers* other than photic cues (Hut et al. 1999). Therefore, before claiming that an organism possesses a circadian locomotor activity rhythm, the endogenous maintenance of a free running rhythm should be demonstrated. Despite various aspects of the Namaqua rock mouse being well-known, basic characteristics regarding its temporal locomotor activity rhythm have not been studied (Skinner and Chimimba 2005). The Namaqua rock mouse is considered nocturnal based on field observations, yet it is possible that non-photic factors within its natural environment is responsible for shaping its purportedly nocturnal behavior and thereby mask its underlying endogenous activity rhythm (Mrosovsky 1999). Such differences in activity patterns have been observed in for example golden hamsters (*Mesocricetus auratus*) and East African root rats (*Tachyoryctes splendens*), where animals exhibit nocturnal activity in a controlled environment, but not in their natural environments (Gattermann et al. 2008; Katandukila et al. 2013). The results of the present study are the first to quantitatively demonstrate the entrainment by the light-dark cycle, of the locomotor activity rhythm of the Namaqua rock mouse and to provide evidence that the species possesses an endogenously controlled nocturnal activity rhythm.

As demonstrated by the actograms, our results revealed a clearly defined phase relationship between the locomotor activity rhythm of the Namaqua rock mouse and the daily light-dark cycle, and furthermore that this relationship is endogenously maintained. When subjected to a 12L: 12D light cycle (LD1 and LD2) all of the animals were almost (~ 99%) exclusively active when it was dark. In addition, an inversion of the light-dark cycle (DL) again produced very high (~96%) nighttime activity, which if given more time would most likely have resulted in an even higher nocturnal percentage. Most rodents are nocturnal, yet trends in their activity rhythms seem to be predominantly constrained by phylogeny and the subfamily Murinae, to which the Namaqua rock mouse belongs, is described as being primarily nocturnal (Roll et al. 2006). Some benefits of a nocturnal lifestyle, particularly in open habitats, include decreased visibility to predators, which thus leaves more time for foraging (Brown et al. 1988; Kotler et al. 1991; Kotler et al. 2002; Rotics et al. 2011).

Although the percentage of activity gives a good measure of the strength of an animal's preference for a particular phase of the day, it does not express how activity is distributed; this can be achieved in the laboratory by the construction of actograms. During entrainment (LD1), activity started only a few minutes after the lights went off and was generally continuous throughout the night with no pronounced peaks or extended times of rest and ended less precisely. It is interesting to note that despite being relieved from many aspects of its daily activity it would normally have in the wild, of which foraging is probably most significant, the Namaqua rock mouse was almost always active throughout the night and had a mean α of 11.53 h under laboratory conditions (during LD1).

Free running rhythms reveal an internal sense of external time and also demonstrate the dependence of the internal clock mechanism on environmental cues for adjusting circadian rhythms to the specific time of the day. In the present study, the activity rhythm was maintained under constant darkness (DD) and a small amount of drift was observed. The τ varied only slightly between individuals and only two mice exhibited a τ marginally above 24 h; the overall mean τ was 23.89 h. This supports the statement that this species is nocturnal since a τ shorter than 24 h in constant darkness is predominantly associated with nocturnal species (Aschoff 1981). Even though the sample size of males versus females was small, sex significantly affected the activity response of the mice in the present study. Females were consistently more active than males and under the DD and LD2 cycles, the differences were significant. This finding is consistent with reports on other rodents in which females generally exhibit higher levels of activity than males, suggested being due to higher levels of estrogen (Lightfoot 2008). Reproductive hormones acutely affect many non-sexual behaviors including locomotor activity and estrogens usually induce an increase in activity; most likely through the oestrogen-α receptor pathway (Ogawa 2003; Lightfoot 2008).

Inversing the light-dark cycle quickly evoked an activity response in the Namaqua rock mouse and revealed that light suppresses activity and darkness induces activity in this species. During the transition of the LD2 light cycle to the inverse DL light cycle, the animals were exposed to 24 h period of light and hence all of the mice postponed activity by a further 12 h. The animals then showed different ways of re-entraining to the DL light cycle since the majority of the mice showed a gradual resetting of the activity offsets

towards the end of the night. Only two mice showed an immediate resetting of the activity rhythm to the DL light cycle. The percentage of nocturnal activity after the mice were fully re-entrained was high (~96%) and given more time, the percentage of nocturnal activity would probably have increased even more. Similar results were obtained in another murid rodent from the Soutpansberg region, the spiny mouse (*Acomys spinosissimus*), that was exposed to experimental conditions similar to that of present study. Activity resetting in spiny mice was also gradual (re-entrainment took approximately 9 days) and the proportion of light versus dark phase activity also did not differ much between the inverse light cycle and the preceding regular 12L: 12D light cycle (Hoole et al. 2012). The rate of circadian re-entrainment is influenced by several factors such as the magnitude and the direction of the phase shift and interestingly, melatonin has been shown to affect the re-entrainment process (Singaravel et al. 1996; Ruby and Heller 1998; Cohen and Kronfeld-Schor 2006). Furthermore, inter-individual differences in re-entrainment may be age-related and even stress-related. In mice, younger individuals have been shown to adjust their circadian timing quicker to shifts in the light schedule than older individuals, and in rats and degus, restraint stress causes a significant delay in re-entrainment (Mohawk and Lee 2005; Davidson et al. 2008; Sellix et al. 2012). It is therefore possible that, in the present study, mice with slower re-entrainment times were either older or more stressed individuals.

The present study revealed that the Namaqua rock mouse possesses a strong nocturnal locomotor activity rhythm that is endogenously entrained by the light-dark cycle. The Namaqua rock mouse was generally active continuously and consistently throughout

most of the night under a normal entrainment period (12L: 12D) and activity onset and offset coincided closely with dusk and dawn, respectively. The activity rhythm persisted under constant darkness with a small amount of drift and with almost all of the activity being expressed during the subjective night. A sudden inversion of the light-dark cycle demonstrated that locomotor activity is prompted by darkness in this species. It is highly probable that this species displays similarly high nocturnal activity in the wild due to the potent effect the light-dark cycle has on its circadian locomotor activity rhythm.

Acknowledgements

The research was supported by a South African Research Chair of Mammal Behavioural Ecology and Physiology awarded to N. C. Bennett. The work was further supported by a collecting permit from the Department of Nature Conservation in Limpopo Province and I. van der Merwe acknowledges a scholarship from the University of Pretoria (UP). We would also like to thank Sonja Faul and Sasha Hoffman for assistance with fieldwork. The authors also thank two anonymous reviewers for their constructive remarks on an earlier draft of this manuscript.

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