Circadian rhythms of locomotor activity in the Lesotho mole-rat, *Cryptomys hottentotus* subspecies from Sani Pass, South Africa

Konrad Schöttner[a, b], Maria K. Oosthuizen[b], Marna Broekman[b] and Nigel C. Bennett[b]

[a]Institute of Zoology, Martin-Luther-University Halle-Wittenberg, 06108 Halle, Germany
[b]Mammal Research Institute, Department of Zoology and Entomology, **University of Pretoria,** Pretoria 0002, South Africa

Abstract

The Lesotho mole-rat is a social subterranean rodent that occurs at altitude in the Drakensberg mountain range. As a consequence of living permanently underground these animals rarely if ever are exposed to light. The visual system of African mole-rats is particularly regressed whereas the circadian system is proportionately conserved. This study investigated the locomotor activity patterns of 12 Lesotho mole-rats maintained under a range of different lighting regimes. The majority (91.7%) of mole-rats entrained their activity patterns to a LD photoperiod of 12L/12D. The mole-rats displayed a monophasic nocturnal activity preference. Under constant dark (DD) most of the mole-rats (83.3%) showed a free running circadian activity pattern with a *tau* of 23.8 h to 24.4 h (mean ± S.E.M.: 24.07 h ± 0.07 h; $n = 10$). The phase of the activity rhythms each mole-rat exerted during the previous LD-cycle did not change when the animals started free-running after being placed in constant conditions. The duration of re-entrainment to a second bout of LD 12:12 amounted to 9.4 ± 2.03 days (mean ± S.E.M., $n = 10$). Eleven mole-rats (91.7%) adjusted their locomotor activity rhythms to an inversed light regime DL 12:12 and displayed significant nocturnal activity preference. The animals required 9.73 ± 2.01 days (mean ± S.E.M., $n = 11$) to adjust to the DL-photoperiod. The Lesotho mole-rat thus possesses a functional circadian clock that responds to a photic *zeitgeber*.

Article Outline

1. Introduction

Biological rhythms are an essential component of life [1]. The generation and molecular timing devices of these rhythms have increasingly become the focus of interest in chronobiological investigations in recent years. The most prominent biological rhythm is the circadian rhythm and it is a distinct feature of a wide range of organisms [1], [2], [3] and [4]. Circadian rhythms are endogenous and self-sustained and they are determined genetically [1], [4] and [5]. In the absence of external cues, the rhythms oscillate or free-run with an intrinsic period (*tau*) of approximately 24 h [6] and [7]. Free-running rhythms reflect the endogenous mechanisms of an internal timekeeping system or biological clock. In mammals, the site of the master circadian clock is the suprachiasmatic nucleus (SCN), located in the anterior hypothalamus dorsal to the optic chiasm [1], [2] and [3]. To provide animals with the ability to anticipate temporal variations of the environment and to maintain an internal time order with respect to that of the external 24 h day, the SCN entrains to periodic environmental stimuli, the so-called *zeitgeber* [10]. The daily light/dark cycle is the most striking environmental *zeitgeber* to which the circadian clock entrains [2], [8] and [9]. The mammalian clock receives photic input strictly from the retina. Photons are captured by photoreceptors in the retina and translated into a neural signal. The information is then transmitted to the SCN via the retinohypothalamic tract (RHT) [1], [6], [10], [11] and [12]. Circadian outputs that regulate distinct rhythms are generated by multiple, single-cell circadian oscillators of the SCN in mammals [11] and [13]. Extra-SCN oscillators are furthermore involved in the circadian timing system, which can be functionally seen as a hierarchical multi-oscillatory system with the SCN on the top [11] and [13]. Since circadian outputs of the SCN cannot be directly measured, overt rhythms (e.g. locomotor activity, temperature) are used as markers of the phase of the biological clock.

The Lesotho mole-rat *Cryptomys hottentotus* subspecies (family: Bathyergidae) is a subterranean, social African rodent occurring in the Sani Pass region in South Africa and the mountain kingdom of Lesotho. African mole-rats spend their entire life under ground and are rarely, if ever exposed to external light as they live in complex burrow systems. Therefore, mole rats show several morphological (reduced body carriage, cylindrical

shaped body and ever growing strong extra-buccal incisors) and physiological (low resting metabolic rate and body temperature) adaptations to their subterranean existence [14] and [15]. Likewise, the visual system of mole-rats has undergone selective changes to adapt to the dark underground environment. Studies of the blind mole-rat (*Spalax ehrenbergi*) established that the circadian system is proportionally expanded, while the image-forming part of the visual system is severely regressed [16] and [17]. Recent findings in African mole-rats have demonstrated that the degree of visual system regression is less than in *S. ehrenbergi*, but still differs to that of sighted mammals [18], [19] and [20]. Several studies have shown that there are varying degrees of photic entrainment of circadian activity rhythms in African mole-rats. The animals display either a predominantly nocturnal or diurnal rhythm, yet within colonies there may be arrhythmic individuals [21], [22], [23], [24], [25] and [26]. These results lead us to suggest that the animals possess a functional circadian system.

The unique subterranean environment of the mole-rat is devoid of regular light information and as a consequence these animals present themselves as ideal models to investigate the circadian system, especially with regard to light as a *zeitgeber*. Since the Lesotho mole-rat is rarely if ever exposed to light, the question then arises as to whether this subterranean mammal can respond to light cues to entrain its daily rhythm of locomotor activity. Therefore, the aim of this study was to investigate whether the Lesotho mole-rat does indeed possess a circadian rhythm of locomotor activity, also if activity rhythms can be entrained to light and finally whether these animals are able to shift their locomotor activity patterns according to shifts in the light cycle.

2. Material and methods

2.1. Animal maintenance

Twelve Lesotho mole-rats, *C. hottentotus* subspecies, weighing 84.5 ± 27.6 g, $n = 6$ at higher altitude and 55.2 ± 20.7 g, $n = 6$ at lower altitude respectively, were caught in grasslands in the Sani Pass region of Lesotho and South Africa utilizing Hickman live-traps [28]. The lower site was situated at 1600 m (29°37′44″S; 29°24′23″E) above sea level and the upper site at 3200 m (29°31′05″S; 29°11′12″E) above sea level. The animals were captured in January 2005 and experiments were commenced in February 2005.

The mole rats, 11 female and 1 male, were housed individually in plastic cages which were provided with wood shavings as nesting material and a plastic tube to sleep in. The experimental room was light and temperature controlled, the animals were maintained on a 12L/12D light regime with lights on at 07h00 before experiments start and room temperature kept around 23 ± 1 °C. Cages were cleaned once every 2 weeks during light cycles and once every 4 weeks during constant conditions (DD). The animals were fed *ad libitum* once a day during light cycles and every second day during constant conditions on chopped sweet potatoes, carrots, apples and grapes at random times so as to prevent entrainment to the feeding regime. The general health of the animals and experimental set up in the climate room was monitored during feeding and cleaning times.

2.2. Experimental model

All experimental animals were exposed to the same series of lighting regimes. The experiment started with a 12L/12D light regime (LD1; lights on at 07h00; 59 days) to determine whether the locomotor activity rhythm of the mole-rat can be entrained to a specific lighting regime. Following this, the mole-rats were exposed to constant conditions (constant darkness, DD; 49 days) to investigate whether an endogenous circadian rhythm of locomotor activity was present in the mole-rats. To assess whether the mole-rats were indeed able to re-entrain to a light cycle, animals were subjected to a second 12L/12D light regime (LD2; lights on at 07h00; 68 days). Finally mole-rats were placed on an inversed 12D/12L light regime (DL; lights off at 07h00; 51 days) to determine if activity was shifted according to changes in the light cycle.

2.3. Activity measurement

Nine cages (60 × 45 × 30 cm) were fitted with infrared captors (Quest PIR internal passive infrared detector; Elite Security Products (ESP), Electronics Line, UK), while three cages (60 × 30 × 30 cm) had running wheels installed (self-manufactured), in addition to the infrared captors. Infrared captors were mounted over the centre of the cage to ensure that movement over the whole area of the cage was monitored. Activity of infrared detectors and running wheels was continuously measured and total amounts of summed activity counts were captured every 60 s by a Mini Mitter computer recording system (Vital View™, Mini-Mitter Co., Inc., Sunriver, OR, USA; www.minimitter.com).

2.4. Data analyses

Actiview© Biological Rhythm Analysis 1.2 software (Mini-Mitter Co., Inc., Sunriver, OR, USA; www.minimitter.com) was employed to generate actograms for visualisation of the data for each light regime. Actograms were double-plotted with consecutive days in a downward sequence. To calculate the amount and percentage of activity during day: Night for LD and DL light regimes, Microsoft Excel was used. One-minute values which were recorded by the Mini Mitter system were summed to 30-min values for consecutive days (LD1: 26 days; DD, LD2, DL: 30 days) for that purpose. Activity profiles of these values were created for LD and DL light regime. In order to detect possible entrainment and diurnal or nocturnal activity preference, chi-square test (χ_{df}^2) was utilized for the analysis of the percentage values of activity during day and night. Data analysis of DD regime was processed in a similar manner to the data collected on LD and DL light regimes. The amount and percentage of activity for subjective day and subjective night was calculated referring to the dark and light period of the previous LD light regime. The program Tau (www.circadian.org) was used to determine the circadian period of free-running rhythms in constant conditions (DD) by means of the chi-square periodogram using summed 6-min values. To detect the time of peak of activity (acrophase) for each lighting regime, the program Acro (www.circadian.org) was applied using the means of the best fitting cosine wave. Summed 6-min values were used for analysis too. Statistical significance was considered by *p*-values less than 0.05. The 14 days after a change in a

light regime was regarded as transition periods and data thereof was not included in the analyses.

3. Results

3.1. General observations

The animals displayed a nocturnal activity preference with monophasic activity patterns during the different light regimes. One mole rat showed arrhythmic locomotor activity throughout the entire experiment. Inter- and intra-individual variation in the patterns of locomotor activity rhythms (Fig. 1 and Fig. 2), total amount of activity counts and time of adaptation to a new lighting regime were observed among individual mole-rats. No wheel running activity was observed by the mole-rats throughout the entire series of experiments.

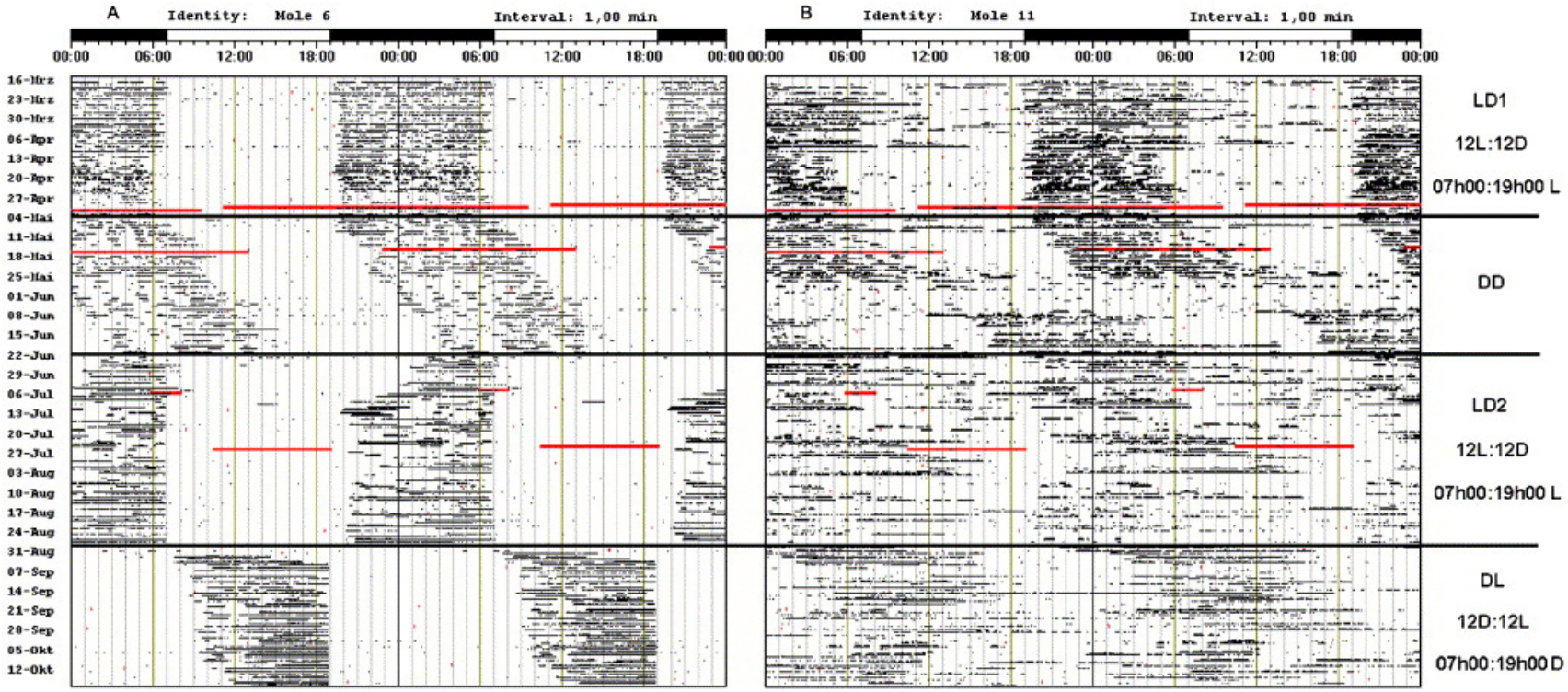

Fig. 1. Double plotted actograms of locomotor activity with data for consecutive days in two individuals of the Lesotho mole-rat during the different light regimes. The black and white bar on top of the actogram represents the respective dark and light phase of the LD-cycle. The individuals displayed good (A) or indifferent (B) entrainment and clear or sloppy locomotor activity rhythms respectively. The actograms illustrate that individuals of the Lesotho mole-rat displayed inter- and intra-individual variability in their locomotor activity rhythms.

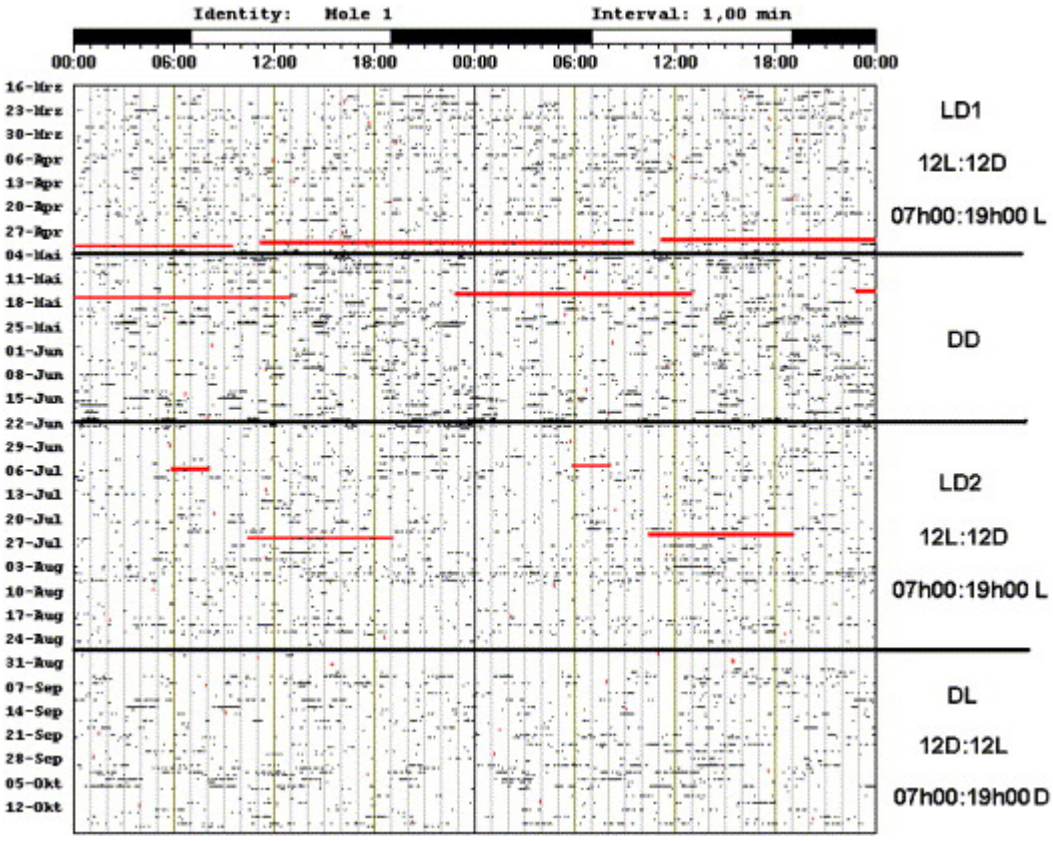

Fig. 2. Double plotted actograms of locomotor activity with data for consecutive days of one Lesotho mole-rat during the different light regimes. The animal displayed arrhythmic activity patterns and no entrainment throughout the entire experiment. It was the only male used in the study. The black and white bar on top of the actogram represents the respective dark and light phase of the LD-cycle.

3.2. Standard photoperiod LD1 (L12/D12)–constant conditions (DD)

Eleven individuals of the Lesotho mole-rat (91.7%) displayed significant nocturnal activity preferences during the first light regime (Fig. 3B,C). One mole-rat (#1) showed more activity during the light phase (Fig. 4), but this animal was excluded from data analyses because it displayed no significant activity preference ($\chi^2 = 2.21$, $p = 0.137$) and no defined locomotor activity rhythm at all (Fig. 3A). The individuals reached a maximum of locomotor activity during the dark phase, with the acrophase occurred at 01h14s ± 00:22 (mean ± S.E.M., $n = 11$). A period of 23.97 ± 0.02 h (mean ± S.E.M.; $n = 11$) was calculated for the activity rhythms. The mean percentage of locomotor activity of the experimental animals during the dark phase was 91.5 ± 0.02%

(mean ± S.E.M.; n = 11), and inter-individual variation referring to the percentage of activity displayed during the dark phase was low (Fig. 4).

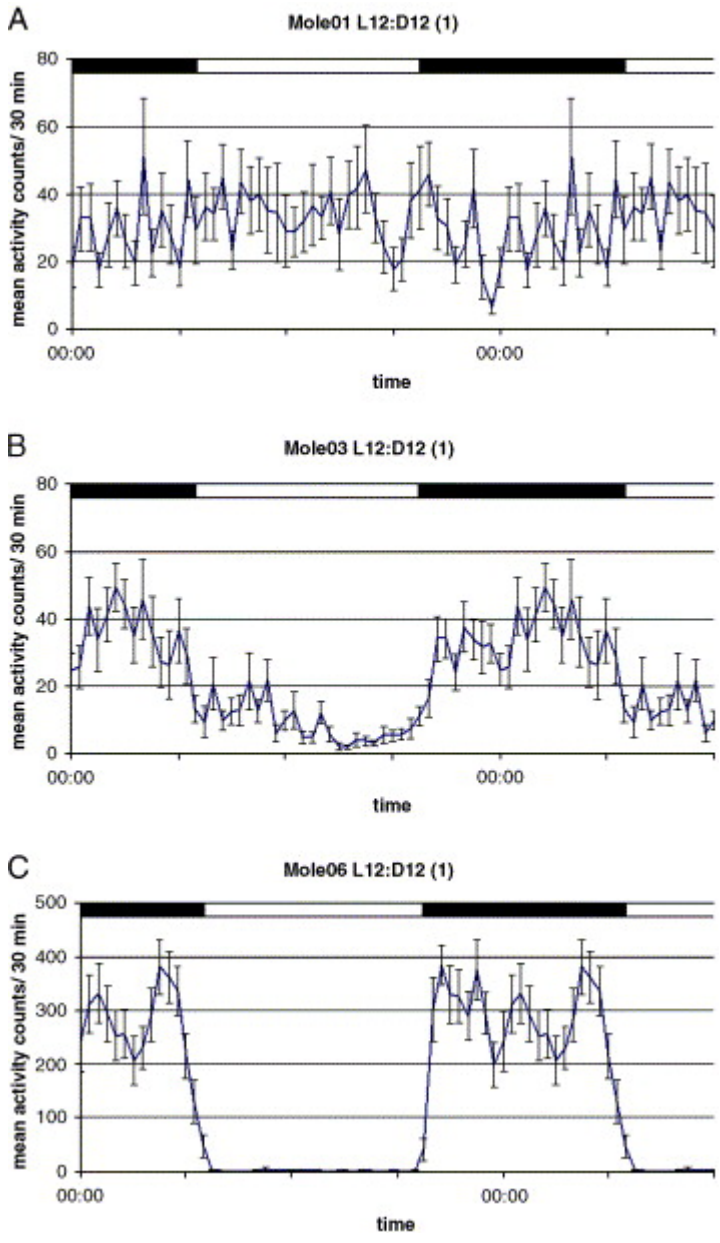

Fig. 3. Activity profiles of individuals of the Lesotho mole-rat displayed either arrhythmic activity patterns and no entrainment (A), indifferent entrainment (B) or clear activity rhythms and good photo-entrainment (C) during the first light/dark (LD) cycle. Black and white bars on top of the graphs representing respective dark and light phase of the LD-cycle. The curve of each graph represents the mean activity counts every 30 min of 26 consecutive days of the first LD-photoperiod. Error bars represent the standard error of mean (S.E.M.).

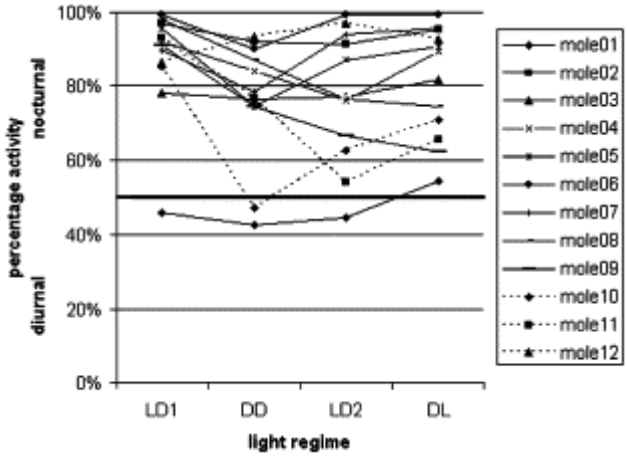

Fig. 4. The graph illustrates the percentage of activity the experimental animals showed during the dark phase/subjective dark phase of each light regime. Inter-individual variability in the percentage of activity displayed in the dark phase is low in the LD1 light regime and increases in the LD2- and DL-light regimes.

The program Tau detected significant endogenous rhythms of locomotor activity during constant conditions (DD) for every mole-rat. However, proper free-running rhythms were determined for only 10 experimental animals by visual estimation of the actograms. Two mole-rats exhibiting sloppy rhythms (#11, Fig. 5B) or rather showing arrhythmic activity patterns (#1, Fig. 2) were excluded from the data analyses used to determine the free-running period. *Tau* (τ) values ranged from 23.8 h to 24.4 h (mean ± S.E.M.: 24.07 h ± 0.07 h; $n = 10$). Ten mole-rats (83.33%) showed significant activity preference during subjective night whereas one mole-rats displayed non-significant activity preference during subjective day (#10: $\chi^2 = 0.30$, $p = 0.586$). When the light regime was changed to constant conditions, animals started free-running with the same phase they displayed during the first LD cycle. Four mole-rats displayed an unusual activity pattern during DD. The period length of their endogenous rhythms changed (e. g. Fig. 5A) and one animal (#11) completely lost the locomotor activity rhythm (Fig. 5B) following a number of days in constant dark.

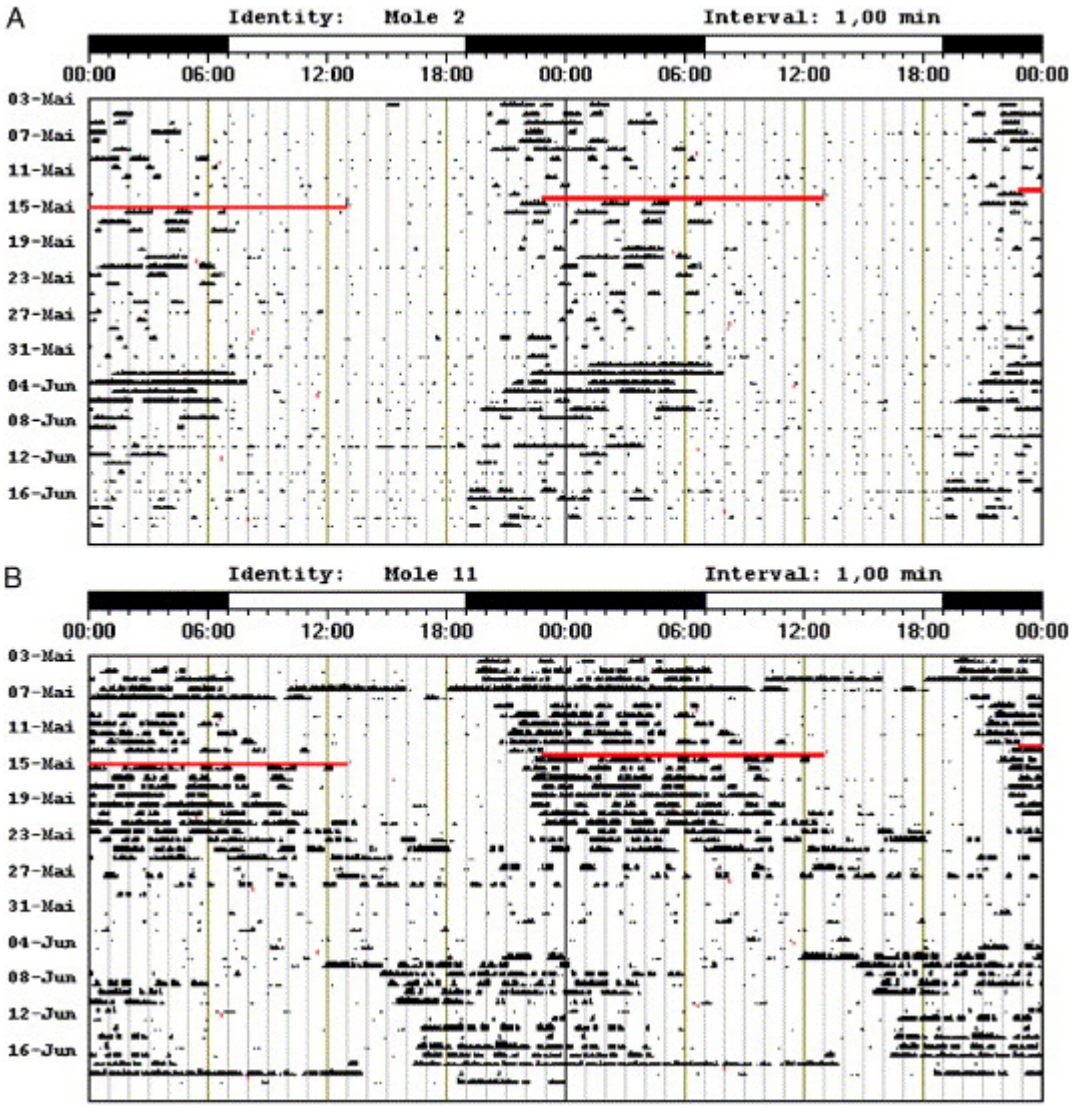

Fig. 5. Double plotted actograms of individuals displaying unusual activity patterns during constant dark. (A) The period of the endogenous activity rhythm of mole #2 changed after some time in constant dark (DD). It is likely that the animals exert unstable rhythms which can even result in losing rhythmicity (B), may be as a consequence of a weak coupling of the circadian pacemaker to its overt rhythms.

3.3. Standard photoperiod LD2 (L12/D12)–inversed photoperiod DL (D12/L12)

The mole-rats readjusted their activity rhythm according to the LD photoperiod, 10 individuals (83.33%) displayed significant and 1 individual (#11) non-significant nocturnal activity preference ($\chi^2 = 0.68$, $p = 0.41$). One mole-rat (#1) remained arrhythmic during LD2 (Fig. 2). The duration of readjustment to the LD-photoperiod required 9.4 ± 2.03 days (mean ± S.E.M., $n = 10$) and the period of the activity rhythms

amounted to 23.99 ± 0.03 h (mean ± S.E.M., *n* = 10). Compared to the initial lighting regime, inter-individual variation as expressed by the percentage of activity during the dark phase was higher (Fig. 4). The mean percentage of activity during the dark phase for all mole-rats together was about 82.8 ± 0.04% (mean ± S.E.M., *n* = 10). However, the acrophase occurred during the dark phase at 01h43 ± 00:46 (mean ± S.E.M., *n* = 10).

Almost all mole-rats adjusted their locomotor activity rhythm to the inversed lighting regime and displayed significant nocturnal activity preference. Only one mole-rat (#1) continued exhibiting arrhythmic activity pattern during DL (Fig. 2). Full adjustment of the activity rhythm according to the inversed light regime required 9.73 ± 2.01 days (mean ± S.E.M., *n* = 11). The degree of inter-individual variation of the percentage of activity during the dark phase did not change considerably compared to the LD2 light regime (Fig. 4). Likewise, the mean percentage of activity displayed by the mole-rats during the dark phase, which amounted to 83.5 ± 0.04% (mean ± S.E.M., *n* = 11), was similar to that of the prior lighting regime. The maximum part of activity occurred during the dark phase with the acrophase was found at 12h44 ± 00:27 (mean ± S.E.M., *n* = 11). A period of 23.98 ± 0.04 h (mean ± S.E.M., *n* = 11) was calculated for the activity rhythms of the experimental animals.

4. Discussion

When presented with a 12L/12D light cycle, individual Lesotho mole-rats exhibited predominantly nocturnal, monophasic locomotor activity patterns, indicating an ability to perceive light and entrain their activity to it. Furthermore, by exposing the experimental animals to constant conditions (DD), endogenous circadian rhythms were revealed, which in turn signifies a functional circadian system. However, the extent of the functionality of the circadian system with light as a primary *zeitgeber* remains enigmatic because noticeable inter- and intra-individual variability in the activity patterns was observed. The spectrum of variability ranged from animals displaying good entrainment of locomotor activity rhythm (Fig. 1A) to animals displaying neither entrainment nor endogenous rhythms of locomotor activity (Fig. 2). These findings (nocturnal activity preference, inter- and intra-individual variation in the activity rhythms) agree with the results found in the highveld mole-rat [24] and the Natal mole-rat [25] which are closely related to the Lesotho mole-rat [15]. Furthermore, inter- and intra-individual variability in activity patterns was observed in studies of the naked mole-rat [23], the Cape mole-rat and Damaraland mole-rat [24] as well as the Mashona mole-rat [26]. Variability in the expressed rhythms may be an indication of a weak coupling of the rhythm to the pacemaker [24] and [26]. However, it may also reflect the possibility that the daily light: dark cycle is not a strong *zeitgeber* in the circadian system of mole-rats due to their subterranean existence. Therefore, it can be argued that there may be a supporting role of non-photic *zeitgebers*, such as temperature and precipitation or social cues that will have considerable influence on the circadian timing system [9] and [14]. For instance, inter- and intra-individual variability of the rhythms of the mole-rats in the recent study increased with progression of the experiment (Fig. 1 and Fig. 4). Interestingly, Bennett [27] did not find a distinct pattern of locomotor activity in a colony of *C. hottentotus hottentotus* which was subjected to constant dim light and similarly Riccio and Goldman

[23] mentioned in their study on the naked mole-rat that most individuals failed to express circadian rhythms of locomotor activity while residing in their colony. However, good entrainment of locomotor activity rhythms was observed in a colony of *C. damarensis* [21]. Therefore, investigations of entire colonies are necessary to find more conclusive results about a potential involvement of social entrainment in the circadian system of mole-rats.

Free-running locomotor activity rhythms of approximately 24 h were observed in 83% of all individuals during constant conditions (DD). The results demonstrate that the Lesotho mole-rat has a functional endogenous circadian timekeeping system or biological clock that is able to express rhythms of locomotor activity. The reason why several individuals displayed free-running rhythms with changing periods during constant dark remains obscure. It is unlikely that “after effects” from previous entrainment were involved [29]. The patterns may simply reflect the fact that the mole-rats display unstable activity rhythms due to a weak coupling between the pacemaker and overt rhythm which can even result in losing circadian rhythmicity (Fig. 5B). However, there was no evidence of splitting in the activity rhythms observed in above-mentioned animals.

Entrainment of the endogenous rhythm of locomotor activity in the Lesotho mole-rat was demonstrated by periods of the activity rhythms that were equal to the 24-h period of the light cycles and a stable phase relationship between the light: dark cycle and the activity rhythms in most animals. Furthermore, the rhythms started free-running with a phase determined by the previous light/dark cycle after exposing the animals to constant conditions. Entrainment as a result of the light has been demnstrated in a number of mole-rat species [21], [22], [23], [24], [25] and [26]. The shift in the activity rhythms according to a shift in the light regime also verify that individuals of the Lesotho mole-rat were able to distinguish between light and dark. Following from this is the notion that subterranean mole-rats may have originally arisen from aboveground ancestors and it can be argued that the circadian system in mole-rats represents a relict trait from an ancestor that did not exclusively exhibit a subterranean life-style [10], [23], [26] and [30]. The closest outgroups for the Bathyergidae include the Cane rats (Thryonomyidae), Dassie rats (Petromuridae) and Porcupines (Hystricidae), all of which live above ground but seek out shelter temporarily in burrows or rock crevices [31].

However, the main question that has to be answered is why subterranean mammals have retained the capability to generate circadian rhythms and maintain a functional circadian system. It is assumed, that the circadian system has evolved on the basis of two selective properties: to provide organisms with the ability to efficiently anticipate cyclic environmental changes, such as periodic changes in the light/dark cycle and furthermore for coordinating internal processes [10], [29] and [32]. Because burrow systems provide a relatively constant habitat for mole-rats where cyclical environmental changes are small if at all present, there is no advantage to maintaining a circadian system for the function to anticipate environmental periodicities, especially the light/dark cycle. But the persistence of an internal timing system and entrainment mechanisms might also have beneficial effects, such as to provide an internal temporal order which may be convenient in the coordination of periodic metabolic processes [10], [23], [29] and [32]. Therefore,

the so-called “intrinsic adaptive advantage” [29] might be one reasonable factor why circadian rhythms in subterranean mammals still exist. Furthermore, it is suggested that the circadian timing system is involved in the modulation of seasonal processes like reproduction. Goldman [10] proposed that there might be a link between the regulatory function of the circadian system and timing of reproductive activity and further that circadian rhythms may play an essential role as a component of the ovulatory cycle. This may have relevance especially for seasonally breeding species.

Although light information cannot be seen as a primary factor triggering seasonal events in strictly subterranean mammals, microclimatic changes in the burrow systems due to seasonal changes in precipitation or temperature can be considered as alternative cues [30]. In conclusion, the Lesotho mole-rat possesses a functional endogenous time-keeping system or biological clock in which circadian rhythms of locomotor activity are manifested under constant conditions. The rhythm can be entrained to light/dark cycles, suggesting that the circadian pacemaker perceives photic information and thus light is a potential *zeitgeber* in *C. hottentotus* subspecies. However, due to inter- and intra-individual variability, the relative importance of light as the major *zeitgeber* still must be ascertained.

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