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Circadian rhythms of locomotor activity in the subterranean Mashona mole rat, *Cryptomys darlingi*

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Abstract

The Mashona mole rat, *Cryptomys darlingi*, is a social, subterranean African rodent that is rarely, if ever, exposed to light, and that exhibits a regressed visual system. This study investigated locomotor activity patterns of Mashona mole rats (n=12) under different light cycles. Activity was measured using either infrared captors (n=8) or running wheels (n=4). The mole rats entrained their activity to a standard (LD 12:12) photoperiod. They displayed either a nocturnal or diurnal activity preference with one bout of activity and one bout of rest. Therefore, as a species, the Mashona mole rat did not show a clear nocturnal or diurnal activity preference. When the LD (12:12) light cycle was inversed, the animals switched their activity, too. Under constant dark (DD), most mole rats (73%) showed a free-running circadian activity rhythm, but under constant light (LL), only some (36%) did. The free-run period of the rhythm (τ) ranged from 23.83 to 24.10 h. The remaining animals were arrhythmic. There was large interindividual and intraindividual variations in the rate and extent of entrainment, time of activity preference, and activity patterns. Possible reasons for the observed variations are discussed. It is concluded that the Mashona mole rat has an endogenous activity rhythm which approximates 24 h, that the mole rat can distinguish between light and dark, and that the endogenous clock utilises this photic information as a *zeitgeber*.

Keywords: Photoperiod; Light; Eye; *Zeitgeber*; Entrainment; Free running; Tau; Masking; Bathyergidae

1. Introduction

The Mashona mole rat, *Cryptomys darlingi*, is a herbivorous, social, subterranean African rodent [1] that lives in a sealed burrow system. The burrow, in which a relatively constant microclimate prevails, provides predator protection and access to food [2,3]. The Mashona mole rat occurs in the Miombo woodlands of Zimbabwe and Mozambique in small familial colonies of between five and nine animals with a hierarchical structure where aseasonal reproduction is limited to the largest male and female in the colony [1,3,4]. An interesting aspect of this species is that the animals are rarely, if ever, exposed to light, because their food, which consists exclusively of bulbs and roots found from the burrow, also provides the required moisture, precluding the need for water [3]. The chance of light exposure is minimal, even when extruding soil from the burrow, which is done by pushing the soil from within against the sealed entrance with their hind feet, thus maintaining a light seal between the animal and the surface (N.C. Bennett, personal communication).

All mole rats are adapted to a subterranean existence and exhibit varying degrees of natural ocular degeneration and visual system regression up to complete visual blindness. Although sharing the same name, Mediterranean mole rats, also called Eurasian mole rats (family Spalacidae), and African mole rats (family Bathyergidae) have apparently evolved independently yet convergently into a subterranean way of life [5–7]. Recent studies have established that the African mole rats (genera *Cryptomys* and *Heterocephalus*) do not exhibit such a high degree of visual system regression as the completely sightless blind mole rat, *Spalax*

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ehrenbergi, but that there are clear differences to that of sighted mammals [5–10]. Consequently, there is a debate in progress with regards to the precise visual abilities of the African mole rats. Up until now, researchers studying bathyergids were of the opinion that they are behaviourally blind [5], but now opinions range from near blindness [8] to basic visual capabilities [5–7,10].

Despite their regressed visual system, African mole rats [11–19] and the blind mole rat [20–24] can perceive photoperiodic changes, indicating that these animals possess functional light–dark photoreceptors in the retina as well as a complete and functional retino-hypothalamic tract. The majority of the above studies investigated locomotor activity rhythms and found that the rodents entrained their activity to photoperiod and exhibited varying degrees of circadian rhythmicity. Entrainment can be defined as the synchronisation of the internal timing system with the external environment [25].

Cooper et al. [26,27] suggest that the photoperiodic system of the blind mole rat has been selectively expanded and progressed, while the 'image-forming' visual system has regressed, thus optimally adapting to life underground. Janssen et al. [28] and Cernuda-Cernuda et al. [29] concurred with Cooper et al. [26,27], suggesting that the retina of the mole rat has undergone evolutionary restructuring to a photoreceptive pineal-like organisation that is used to mediate light-dependent entrainment of circadian and circannual rhythms. Lovegrove et al. [12] surmised that the white head patch, characteristic of some mole-rat species, may function as a photic window, facilitating increased light reception to the pineal gland; however, the current conviction is that mammalian photoreceptors occur only in the eye [6,30]. Thus, it has become clear that photoreception is not restricted to vision in mammals [30].

Circadian rhythms, which, by definition, are endogenous, have been found in virtually all organisms that have been examined to date [22]. An internal time-keeping system, the endogenous pacemakers, also termed biological clocks or oscillators, drive the circadian rhythms of behaviour, physiology, and metabolism in organisms [31]. In mammals, the suprachiasmatic nucleus (SCN) within the hypothalamus appears to be the site of the master pacemaker [31–33]. Ordinarily, the clock is entrained to a timing cue, also termed *zeitgeber*. The environmental irradiance change between day and night, which is processed via the retinal input pathway, is the main photic cue that mammals use to entrain their circadian rhythms [21,30,32,34]. Nonphotic stimuli such as temperature [21], circulating melatonin, locomotor activity, periodic feeding [33], forced activity, and social cues [25] can also entrain circadian rhythms. In the absence of timing cues, the clock of the SCN free-runs or oscillates with an approximate 24-h periodicity, expressing its endogenous circadian period τ [25]. However, the clock can also be masked by the *zeitgeber*. Masking is defined as an immediate stimulus–response reaction, as opposed to the synchronisation of the clock to the stimulus, and can enhance (positive masking) or suppress (negative masking) the variable that is being observed [35]. It has become increasingly apparent that circadian rhythms are necessary for life, that probably all the physiological systems are under the influence of circadian neural signals [36], and that mammals have multiple circadian oscillators, neural and other, throughout their body that can independently synchronise to different environmental stimuli [37].

Whilst it is evident that photoreceptive mechanisms are used to regulate the clock [30], neither rods nor cones are required for mammalian photoentrainment [38]. But our understanding of these circadian light detection sensory systems within the eye remains superficial [30] and the mechanism underlying the circadian rhythmicity of the master pacemaker in the SCN is not yet unravelled [33]. Currently, it is thought that the core molecular structure of the circadian system consists of a number of clock genes and their products [39].

Circadian systems are believed to have evolved because they provide organisms with the ability to anticipate, and thus prepare for, relatively predictable environmental changes that are associated with the day–night cycle (e.g., summer and winter) [32]. The unique subterranean environment of the mole rats renders these rodents of special interest for studies on the mammalian circadian system [22,24,32]. In addition, the microphthalmic condition of the visual system of these animals provides an exceptional opportunity to investigate the chronobiology and sensory biology of the mole rat. Since the daily light–dark rhythm is absent in the environment of the African mole rats, it is thought that other environmental factors probably have an overriding influence in modulating circadian as well as seasonal behaviour [3]. Begall et al. [40] have intimated that studies of activity patterns on subterranean rodents are necessary to identify both the ultimate factors, which maintain rhythmicity in a constant environment, and the proximate ones, which cause entrainment in nature. Such studies may ultimately aid in the understanding and identification of the photoreceptors that regulate circadian responses in mole rats and other mammals [41]. Even though there have been a considerable number of studies on the circadian systems of nonfossorial rodents, and some on the blind mole rat, the knowledge on African mole rats is less extensive.

In view of the above considerations, the objectives of this study were: (1) to determine whether the Mashona mole rats have circadian locomotor activity rhythms; (2) to establish if they entrain to light; and (3) if there are locomotor activity patterns, to ascertain their response to different photoperiods.

2. Materials and methods

2.1. Animal maintenance and experiment room

The Mashona mole rats that were used in this study were descendants of animals caught in the wild in Zimbabwe near

Harare at Goromonzi (17°S, 31°E) in 1992. The experimental room was temperature- (25±1 °C) and light-controlled. The room was fitted with a corridor and double doors, thereby facilitating access to the room when the laboratory lighting differed from the photoperiod of the experimental room. Access to the experimental room was restricted so that the room environment remained constant and the mole rats experienced minimal noise and disturbance. The animals were kept individually in plastic containers (~70×40×40 cm) and were provided with wood shavings as nesting material; bedding and containers were cleaned regularly. A piece of clear Perspex piping that the animal could enter was provided in each container.

Under the acclimatisation LD photoperiod prior to the experiment, it was observed that when the mole rats did not have a large-enough fresh food reserve, they showed an increase in locomotor activity, indicating that the animals were searching for food. Consequently, the mole rats were fed ad libitum once a day with large food portions of chopped sweet potato, gem squash, carrots, apples, and grapes to avoid activity that was related to a scarcity of food. The time of feeding was varied each day to prevent entrainment of the animals to the feeding regime. If feeding fell within the time period when the mole rats were in the dark, then a weak red-filtered headlamp with a light intensity of <0.125 lux was used as sparingly as possible to minimise animal light exposure. At no time during the dark phases were the mole rats exposed to higher levels of light. No free water was provided. The general health status of the animals and the functioning of the equipment were monitored daily when feeding the mole rats. The sex and number of animals used were dependent upon availability. The Animal Ethics Committee of the University of Pretoria approved the protocol for this study (permit no. EC-020718-01).

2.2. Experimental model

The Mashona mole rats (n=12; 10 males, 2 females) were all subjected to different sequential lighting regimes for a total experimental period of approximately 10 months (314 days) (Fig. 1). During the light and dark phases, the mole rats were exposed to a light intensity of approximately 500 and 0.025 lx, respectively. These lux values were chosen because they were the closest values (in our laboratory) possible to natural daytime and nighttime light. Henceforth, 12:12 LD, 12:12 DL, constant dark, constant light, and 3:3 DL masking will be referred to as LD, DL, DD, LL, and masking, respectively. During the study, the locomotor activity of all animals was measured by means of either infrared captors that recorded any locomotor movement (n=8) [Quest PIR internal passive infrared detector; Elite Security Products (ESP), Electronics Line, UK] or running wheels that measured wheel revolutions (n=4) (self-manufactured). These two methods allowed the determination of whether locomotor activity is a rhythmic or arrhythmic

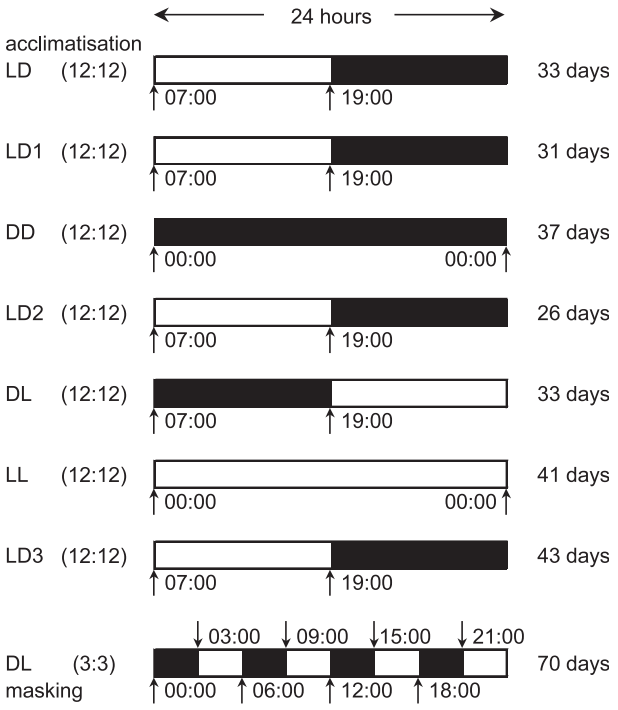

Fig. 1. Experimental model of the study depicting the sequential photoperiods that the Mashona mole rats were subjected to (top to bottom) and the number of days under each photoperiod. L=light (~500 lux) is shown by a white bar, while D=dark (~0.025 lux) is shown by a black bar. The numbers in brackets refer to the number of hours of either light or dark, and the times refer to local time when the lights were switched either on or off. LD (acclimatisation) was used to expose all mole rats to the same photoperiod before starting the experiment.

event. The data are not comparable in terms of absolute values, but does allow some comparison by means of relative frequencies of activity.

2.3. Locomotor activity measurement

The mole-rat individuals were numbered from 1 to 12 with numbers 11 and 12 being females. Clear Perspex running wheels with a diameter of 15 cm and a magnetic switch measured the locomotor activity of animals 5–8. The locomotor activity of the remaining eight mole rats was measured using infrared captors. Movements such as twitching in sleep, heavy breathing, and grooming did not trigger the infrared captors. The running wheels were placed on the shorter side of the container, and the infrared captors were attached to the centre of a bar crossing the width of the cage, thereby enabling detection of locomotor activity across the whole width and length of the cage. The mole-rat activity was measured continuously and the summed measurements were recorded every 60 s by the computer-run VitalView Mini-Mitter system (www.minimitter.com).

2.4. Data analysis

The ActiView (www.minimitter.com) software package was utilised to generate double-plotted actograms of the

activity recordings for visualisation of the data. The vertical axis of the actograms displays consecutive days and the horizontal axis displays 48 h with every 24-h interval presented twice. The ClockLab software package (www.actimetrics.com) was used to calculate the amount of activity during the light and dark phase(s) of the LD, DL, and masking lighting regimes by converting the visual data into numbers. Transition phases that would distort these calculations were excluded or corrected for in ClockLab. Similarly, in DD and LL, the amounts of activity for subjective night and subjective day (the period that an individual mole rat perceives as night or day under constant conditions) were calculated. This was done by using the dark and light periods of the previous lighting regime as subjective night and day, respectively. In animals that exhibited circadian rhythms under constant conditions, τ was determined and corrected for in the calculations with ClockLab.

The percentage locomotor activity during the light and dark phases of each light cycle was calculated and the chi-square test (χ^2_{df}) was employed to determine possible entrainment and/or nocturnal or diurnal activity preference. Entrainment was assumed when there was significantly more locomotor activity in either the light or dark phase of the photoperiod, while nocturnal and diurnal activity preferences were used to refer to the phase of the photoperiod to which the animal was entrained. However, entrainment was considered most probable when corresponding shifts in locomotor activity and photoperiod occurred in an individual, while entrainment was considered conclusively demonstrated when a free-running animal under constant conditions started to drift from the same phase angle to which it had synchronised under the preceding light–dark cycle [18]. Animals that did not entrain were considered arrhythmic. For the DD and LL photoperiods, the same measurements were calculated but utilised subjective day and subjective night instead of light and dark. The nocturnal or diurnal preference of all 12 animals as a group, as well as the entrainment of all 12 animals as a group, were determined for every light cycle using the numbers of diurnal and nocturnal, and entrained and not entrained animals, respectively (χ^2_{df}). Total locomotor activity of the animals recorded with running wheels was compared to the total locomotor activity of the animals recorded with infrared captors by means of the Mann–Whitney U test (U). To establish an individual mole rat's diurnal or nocturnal activity preference throughout the whole experimental period, the light and dark percentages as calculated by ClockLab were taken into consideration but more weight was given to the visual data on the double-plotted actogram. The latter data provide a more accurate pattern than when relying only on the numerical data, since the numerical data cannot describe a mole rat's activity pattern as well as the human investigator (H.M. Cooper, personal communication).

3. Results

3.1. General observations

The Mashona mole rats displayed a monophasic activity pattern, with either a diurnal or nocturnal locomotor activity preference. However, one animal displayed a biphasic activity pattern under LD2 and DL (Fig. 2).

There was no apparent diurnal or nocturnal locomotor activity preference in the Mashona mole rat as a species. When the active phase for each animal over the whole experimental period was determined, five animals (41.7%) showed diurnal preferences (animals 1, 8, 9, 10, and 12) and five (41.7%) showed nocturnal preferences (animals 2, 3, 4, 5, and 11). The preferred time of activity of animals 6 and 7 (16.7%) was inconclusive.

The Mashona mole rat exhibits sloppy rhythms because the rhythms are characterised by a certain degree of lability with respect to their overt expression, making visual assessment difficult (Fig. 3). There were even some instances where no obvious activity rhythms could be detected visually from the double-plotted actograms. However, when the ClockLab-computed numbers representing activity were analysed, there were only two animals that did not show a significant diurnal or nocturnal preference throughout the whole experiment (χ^2_1=1.553, P=0.2127; χ^2_1=1.234, P=0.2667). Activity onsets were commonly clearer and more precise than the corresponding activity offsets. Generally, the mole rats were unable to entrain to a

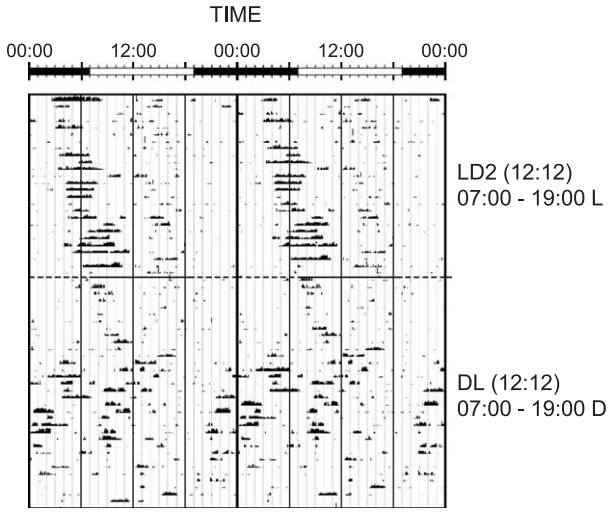

Fig. 2. Double-plotted actogram (see Materials and Methods and Data Analysis) of the locomotor activity of Mashona mole rat 9 under the standard photoperiod LD2 and the inverse photoperiod DL. The dotted part of the line dividing the two photoperiods indicates the light phase of the light cycle underneath it. This animal displayed a biphasic activity pattern under both LD2 and DL. The actogram also shows how the activity pattern of this mole rat slowly drifted from left to right throughout the whole LD2 photoperiod, and from the right to the left under DL. The reversal of drift direction brought about by the switch in light cycle supports the premise that the mole rat was only able to slowly adjust its period of activity to the new photoperiod.

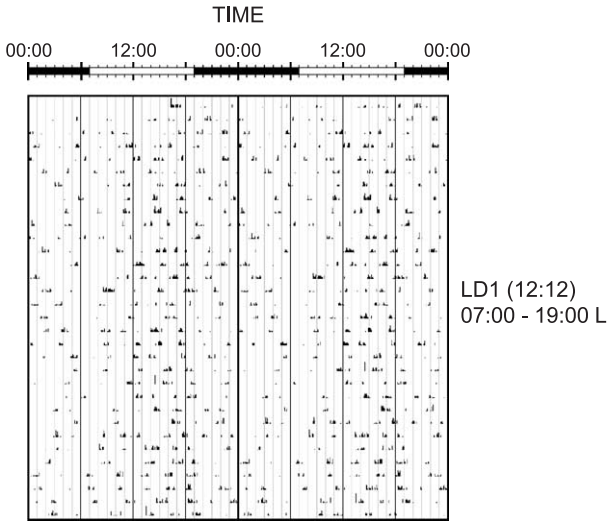

Fig. 3. Double-plotted actogram of the locomotor activity of Mashona mole rat 1 under the standard photoperiod LD1, displaying a sloppily entrained rhythm. Even though the mole rat shows a diurnal preference (χ^2_1=78.207, P<0.0001) for its locomotor activity, there is some activity outside the light phase. Moreover, the activity does not have a distinct activity onset or offset. It can be concluded that the displayed rhythm is not due to masking because this animal's rhythm started to drift under DD from the same phase angle to which the animal was entrained under LD1.

new lighting regime rapidly, and the animals frequently drifted for a period of approximately 10–14 days and even up to a month (Fig. 2), before adjustment occurred.

There was no difference in the total locomotor activity of mole rats in cages with running wheels and those in cages without running wheels (U=420.5, n_{wheel}=24; n_{sensor}=48, P=0.0633). Therefore, there is a negligible amount of activity that the running wheel does not pick up because the animal is not active in the wheel.

3.2. Standard photoperiod—constant dark photoperiod (LD1–DD)

Sloppy locomotor activity entrainment was observed under LD1. However, entrainment of the locomotor activity did occur in all 12 mole rats (χ^2_1=12.000, P<0.0005). Nine mole rats (75.0%) displayed a nocturnal activity preference and the remaining three (25.0%) showed a diurnal preference. There was no significant diurnal or nocturnal preference under LD1 for the activity of all 12 mole rats combined (χ^2_1=3.000, P=0.0832). From a descriptive and visual point of view, only five animals (41.7%) exhibited distinct and clear rhythms that were not overtly sloppy.

Due to health reasons, there were insufficient data for one individual under DD, leading it to be excluded from the analysis of this photoperiod. When the light cycle was switched to DD, eight (72.7%) of the 11 mole rats exhibited distinct free-running circadian rhythms of locomotor activity (Fig. 4), but the remaining three (27.3%) were arrhythmic. This arrhythmia under DD is unusual for rodents. There was little variation in DD τ values, which ranged from 23.90 to 24.10 h (mean±S.D.: 23.99±0.062 h). With the exception of one animal, all free-running animals under DD displayed an increase in the amount of activity during the subjective day, irrespective of whether τ, which determines the angle and direction of free running, was smaller or larger than 24 h.

3.3. Standard photoperiod—inversed standard photoperiod (LD2-DL)

Animals reentrained to the standard photoperiod when the photoperiod was switched back to LD2 from DD. The mole rats also slowly reentrained their locomotor activity rhythms when the standard photoperiod was inversed from LD2 to DL, with some of the animals taking the entire photoperiod to readjust (Fig. 2). Under the LD2 and DL photoperiods, locomotor activity reentrainment occurred in 11 of the 12 mole rats (91.7%) (LD2 and DL: χ^2_1=8.333, P<0.0039), showing significant entrainment to standard lighting regimes.

Of the 11 mole rats, six (54.5%) showed a nocturnal activity preference and five (45.5%) a diurnal activity preference under LD2. There was no significant nocturnal or diurnal preference under LD2 for this mole-rat species (χ^2_1=0.091, P=0.7630).

However, under DL, the animals displayed a nocturnal activity preference (χ^2_1=4.455, P<0.0348), with two of the 11 mole rats (18.2%) showing a diurnal preference and nine (81.8%) a nocturnal preference. Two animals displayed entrainment to the DL regime after 3 weeks of arrhythmic activity, but the majority of the animals drifted through nearly the whole DL cycle trying to inverse their activity rhythms (Fig. 2). Only one animal (animal 3) was able to

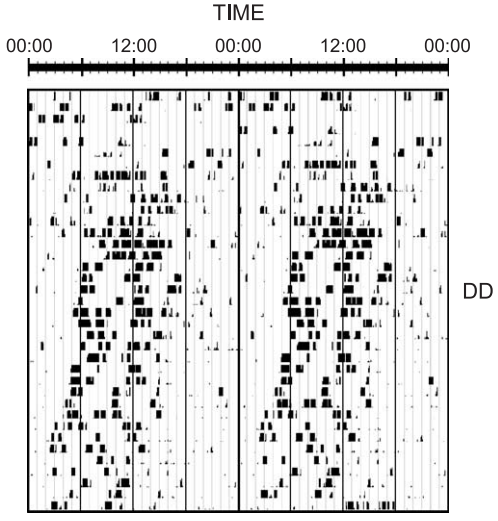

Fig. 4. Double-plotted actogram of the locomotor activity of Mashona mole rat 7 under the constant dark photoperiod DD. This animal displayed a distinct free-running endogenous locomotor activity rhythm with τ=23.90 h. The preferred time of activity was subjective day (χ^2_1=4716.061, P<0.0001).

switch immediately from the LD2 to the DL photoperiod (Fig. 5).

Mole rats 4 and 12 displayed unusual behaviour under the reversal of the photoperiod, with their preferred activity time remaining the same under both lighting regimes, irrespective of whether that time was in the dark or the light. Animal 4 showed indistinct rhythms under the LD1, LD2, DL, and LD3 lighting regimes, and was arrhythmic under DD, LL, and masking.

3.4. Constant light photoperiod—standard photoperiod (LL–LD3)

Arrhythmic locomotor activity rhythms and circadian rhythms were found under constant light and reentrainment occurred under LD3. Due to health reasons, there were insufficient data for one individual under DD, leading it to be excluded from the analysis of this photoperiod. Out of the 11 remaining mole rats, four (36.4%) displayed endogenous free-running rhythms (Fig. 5), while the other seven animals (63.6%) displayed arrhythmicity. One of the seven mole rats exhibited a rhythm for 16 days before becoming arrhythmic, while the other six became arrhythmic within approximately 8 days or showed an immediate breakdown of rhythmicity. Three of the four free-running animals (75.0%) displayed a subjective night activity preference, while one (25.0%) showed a subjective day preference. Once again, the Mashona mole rat as a species did not have a preferred activity time (χ^2_1=1.000, P=0.3173). Under LL, no splitting of the rhythms was observed, and τ ranged from 23.83 to 24.07 h (mean±S.D.: 23.96±0.085 h). With the exception of one animal, all free-running animals under LL displayed a decrease in the amount of activity during the subjective day, irrespective of whether τ, which determines the angle and direction of free running, was smaller or larger than 24 h.

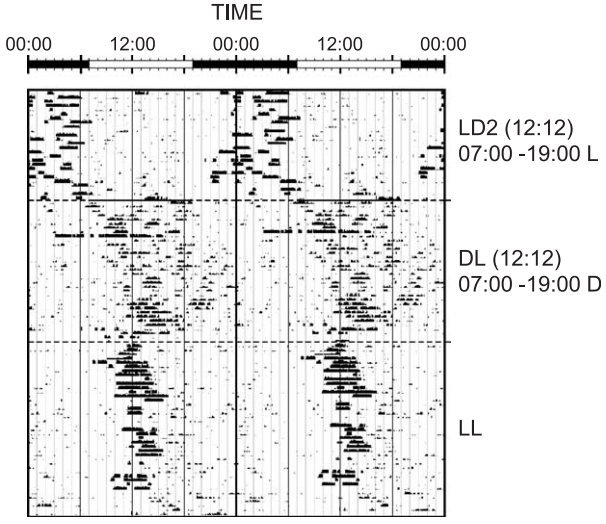

Fig. 5. Double-plotted actogram of the locomotor activity of Mashona mole rat 3 under the standard photoperiod LD2, inverse photoperiod DL, and constant light LL. The dotted part of the line dividing the photoperiods indicates the light phase of the light cycle underneath it. This mole rat was the only individual that displayed an immediate activity switch when the photoperiod changed from LD2 to DL. Under the LL photoperiod, this animal showed a distinct free-running endogenous rhythm of locomotor activity with τ=24.07 h. The preferred time of activity was subjective night (χ^2_1=3126.525, P<0.0001).

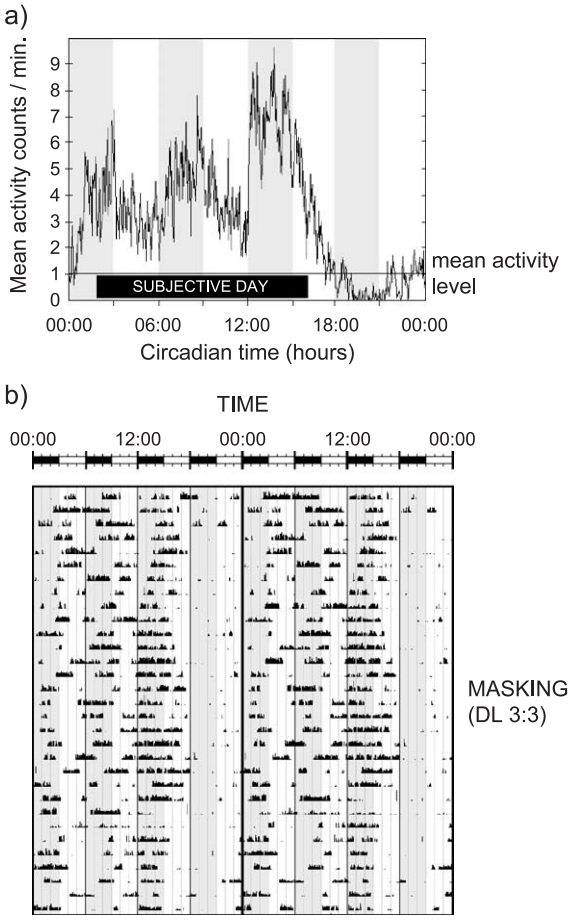

Fig. 6. Activity profile (a) and double-plotted actogram (b) of the locomotor activity of Mashona mole rat 12 for days 230–260 under the 3-h dark, 3-h light masking photoperiod. The grey and white backgrounds represent the respective dark and light phases of the photoperiods. This mole rat displayed masking while maintaining its monophasic endogenous circadian activity rhythm (τ=23.8) with the preferred time of locomotor activity being subjective day. During this period, the mole rat's subjective day was from 02:00 to 16:10 h. There are two light and dark phases that fall approximately within the animal's subjective day (03:00–09:00 and 09:00–15:00 h). Light is significantly suppressing locomotor activity during the two subjective day active phases (03:00–09:00 h, χ^2_1=10.741, P<0.0010; 09:00–15:00 h, χ^2_1=69.045, P<0.0001), thus showing that light pulses had the power to override the circadian rhythm control. Furthermore, mean locomotor activity in the dark is 1.4 times higher than in the light. During the mole rat's subjective night, there was minimal variation in activity between the light and dark periods, with the dark level being 1.09 times higher than the light one. However, this is most unusual behaviour, since animal 12 displayed a significant diurnal activity preference under standard photoperiods LD1, LD2, and LD3 (Fig. 8), leading to the expectation that dark and not light pulses would suppress the activity.

When subjected to the switch in lighting regime from LL to LD3, all 12 animals reentrained (χ^2_1=12.000, P<0.0005) to the standard photoperiod. Five animals (41.7%) showed a

diurnal activity preference and seven (58.3%) a nocturnal one. Therefore, there was no general preference for time of activity in the Mashona mole rat under LD3 (χ^2_1=0.333, P=0.5637).

3.5. Masking

Only two of the 12 mole rats (16.7%) responded to the masking light cycle. Interestingly, these two animals were the only two females in the experiment. One animal showed masking and another one appeared to entrain to the masking light cycle. This entrainment can also be interpreted as masking but with no expression of circadian rhythm (Figs. 6a and b, and 7a and b).

From both visual assessment of the actograms and ClockLab, it became clear that the other 10 individuals, which were all males, either displayed drifting endogenous rhythms (n=5, 41.7%, τ<24.0 h) or became arrhythmic (n=5, 41.7%).

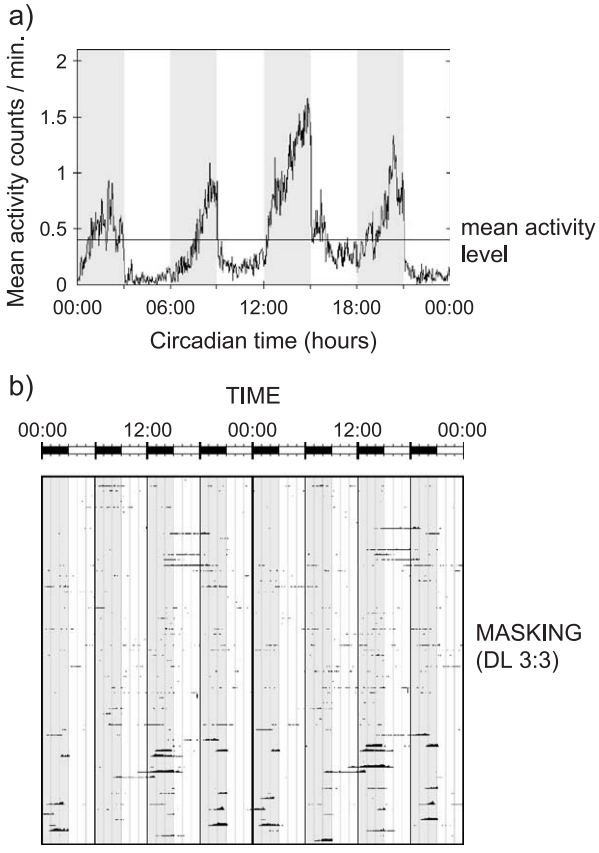

Fig. 7. Activity profile (a) and double-plotted actogram (b) of the locomotor activity of Mashona mole rat 11 for days 204–281 under the 3-h dark, 3-h light masking photoperiod, displaying entrainment to the masking photoperiod. The grey and white backgrounds represent the respective dark and light phases of the photoperiods. This mole rat showed no circadian activity rhythm over the period of four of these 6-h photoperiods, but it significantly adjusted its time of locomotor activity to the four dark periods in the 24-h day (0–6 h: χ^2_1=50.261, P<0.0001; 6–12 h: χ^2_1=13.703, P<0.0002; 12–18 h: χ^2_1=46.960, P<0.0001; and 18–0 h: χ^2_1=65.108, P<0.0001), thereby displaying entrainment to the short masking photoperiod. In the last third of the actogram, this is most visible. The mean activity during all four dark phases is 3.2 times higher than the mean activity during the light phases.

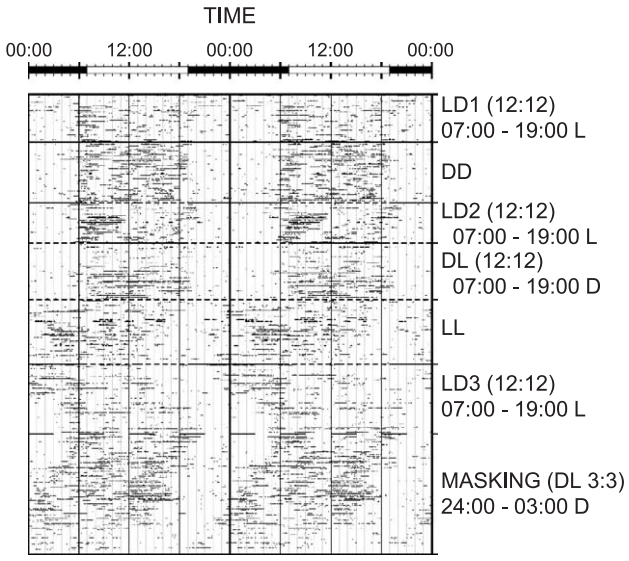

Fig. 8. Double-plotted actogram of the unusual locomotor activity of Mashona mole rat 12 throughout the whole study under all photoperiods. The dotted parts of the lines dividing the photoperiods indicate the light phase of the light cycle underneath it. Mole rat 12 appeared to be entrained to the LD1 photoperiod with a significant diurnal preference (χ^2_1=405.430, P<0.0001), but it maintained this stable, monophasic activity rhythm irrespective of the photoperiodic changes that it was subjected to (constant dark DD, standard photoperiod LD2, and inverse standard photoperiod DL). However, under constant light LL, this rhythm changed and the mole rat displayed a clear, apparently free-running circadian activity rhythm with τ=23.97 h (χ^2_1=18.143, P<0.0001), and under standard photoperiod LD3, it reentrained slowly to the previously displayed diurnal preference (χ^2_1=11.191, P<0.0008). This individual's masking behaviour appears in Fig. 6.

3.6. Unusual behaviour patterns

Mole rat 12 showed unusual behaviour throughout most of the study in that it only sometimes adjusted to the prevalent photoperiods (Fig. 8). Further unusual behaviour was described at the end of the LD2–DL section and in Fig. 6.

4. Discussion

4.1. Temporal activity distribution

Individual Mashona mole rats displayed both diurnal and nocturnal locomotor activity preferences with predominantly monophasic activity patterns, indicating that they are able to distinguish between light and dark. However, there was noticeable interindividual and intraindividual variability in both the activity pattern and diurnal or nocturnal activity preference. Even though several activity rhythms visually appeared to be arrhythmic in the actograms, there were in fact only two animals that did not show

significant diurnal or nocturnal preference throughout the whole experiment. Despite that, there was no clear indication of a diurnal or nocturnal activity preference for this mole-rat species. The facts that light suppressed activity under the masking photoperiod, and that double the number of animals displayed an endogenous locomotor activity under DD, as opposed to LL, could indicate a possible nocturnal activity preference.

On one hand, the finding that no preferred time of activity was found for the Mashona mole rat conforms to studies conducted on other social African mole rats. A lack of distinct diurnal or nocturnal activity preference was found in colonies of the common mole rat, *Cryptomys hottentotus hottentotus* [42], *Cryptomys hottentotus* (assumed be Natal mole rat, *Cryptomys hottentotus natalensis*, due to their capture location) [11], and the naked mole rat, *Heterocephalus glaber* [15]. However, Hickman [11] does state, that there may have been slightly less activity during the light phase than the dark phase. On the other hand, this finding is in contrast to what has been described in other mole-rat species. Nocturnal activity preferences were found in individually kept Natal mole rats, *C. hottentotus natalensis* [19] and Highveld mole rats, *Cryptomys hottentotus pretoriae*, but diurnal activity preferences emerged in colonies [12] as well as in individually kept Damaraland mole rats, *Cryptomys damarensis* [18]. The solitary Cape mole rat, *Georychus capensis*, is clearly nocturnal [13,14,18] and the blind mole rat appears to be diurnal [22,24]. With the exception of the common mole-rat colony in the study of Bennett [42], which was subjected to constant dim light, all other mole rats were subjected to some form of 24-h LD photoperiod. It has been suggested that social mole rats are less capable of exhibiting clear nocturnal or diurnal activity rhythms than solitary ones [18]. Many of the abovementioned studies infer the time of the preferred activity from the preference that the majority of animals display. This indicates that few mole-rat species appear to show exclusively diurnal or nocturnal activity patterns. Furthermore, the variability displayed by the Mashona mole rats in this study has also been stressed by other authors in both solitary and social species [15,18,19,22,24]. This variability could be indicative of weak coupling between the pacemaker and its overt rhythm [18].

The reasons why some mole-rat species are more active during the light phase of a photoperiod and others during the dark phase remain obscure. It is possible that evolution caused mole rats to favour activity in the opposing period of a diurnally or nocturnally active predator, but Hickman [11] does not believe that nocturnal or diurnal predators would exert more selective pressure over other nonnocturnal or diurnal mole-rat predators. It may also simply be that these differences reflect the fact that light is an anomalous occurrence in the mole-rat environment [18]. However, diurnal and nocturnal activity preferences within a colony of the social Mashona mole rat may well have advantages for burrow protection and, hence, colony defence. A subterranean burrow can be breached at any time of the day or night and the probability of detecting conspecifics and predators, particularly snakes, that have entered the burrow is greatly increased by having animals active throughout the full 24-h cycle. Thus, this would require a mole rat to be flexible with regards to the time of its activity, as the time at which the individual is active might be imposed by the colony.

4.2. Entrainment of locomotor activity

Under standard LD photoperiods, practically all the Mashona mole rats showed significant entrainment of locomotor activity. Twice (LD1, LD3) all mole rats entrained and twice (LD2, DL) only one individual (8.3%) did not entrain. Once again, there were large variations in the rate as well as extent of entrainment. When the LD photoperiod was inversed, the activity rhythm was shifted accordingly in most animals (83.3%), but slowly. Just one animal shifted its activity rhythm immediately. The phase shift of locomotor activity with a corresponding shift in the photoperiod showed that the mole rats were entrained to the light rather than some other uncontrolled *zeitgeber*. Therefore, the Mashona mole rat is able to distinguish the difference between light and dark, and has a biological clock that integrates this photic information and utilises it as a *zeitgeber* to entrain locomotor activity.

There are numerous possible reasons that may explain the observed variations in the rate and extent of entrainment in this species. They may once again be indicative of a weak link between the endogenous clock and its output. Moreover, it is possible that nonphotic cues, such as temperature [22], may play an important supporting role in the entrainment of subterranean mole rats [3].

Oosthuizen et al. [18] found that social mole rats were slower in their adjustment to new photoperiods, and that they had a lower rate of locomotor activity entrainment than solitary ones. They suggest that this could be a result of individually housed social mole rats behaving aberrantly because of their separation from conspecifics, or that the social mole rats depend on social entrainment cues [18]. The above suggestions only partially correspond with conclusions of the present study, in which the Mashona mole rat indeed takes long to adjust to an inversal of photoperiod, but where entrainment is high. It is suggested that a study, which tests the hypothesis that a social factor is involved in these differences, is conducted.

Sometimes animals are screened for rhythm displays before they are included in chronobiological studies. The mole rats that were used here were purposely not screened for rhythmicity before the experiment began, so as to obtain unbiased data that are representative of locomotor activity in the Mashona mole rat. Consequently, the conclusions drawn in studies that only included animals that had displayed good rhythms during prescreening [15,16] cannot be

compared directly with studies where no screening was practiced.

It has also been suggested that extended light exposure of mole rats may cause some degree of retinal degeneration that could reduce photosensitivity [15]. However, findings of both Nemec et al. [5] and Peichl et al. [6] indicate that this is not the case. More plausibly, the Mashona mole rat does not need to entrain to photic cues as it is an aseasonal breeder [1]. This hypothesis is supported by the finding that the also aseasonally breeding Ansell's mole rat, *Cryptomys anselli* (formerly Zambian mole rat, *C. hottentotus hottentotus*, or *Cryptomys* sp.), lacks retinal projections to the bed nucleus of the stria terminalis, which is implicated in photic control of seasonal reproduction and thermoregulation [5]. Lastly, the observed photic entrainment with large interindividual and intraindividual variations may be explained by the Mashona mole rat having become secondarily subterranean over evolutionary time, leading some of the various structures involved in photic transduction and its subsequent interpretation to regress but not yet to entirely lose their function. This supposition is underscored by the finding that the visual system of *Cryptomys* occupies the intriguing intermediate position between that of the blind mole rat and sighted mammals [5,6,8,9].

There are several other possible reasons why there could be large interindividual and intraindividual variation in entrainment. Abnormalities in the clock genes appear to play an important role in photoperiodic perception and expression [43]. It has been found that aberrations in a clock gene in humans cause sleep disorders [44], that some mouse *Period* genes are necessary for shifting the wheel-running activity [45], and that only a single *mPer* or *Chryptochrome mCry* gene is not sufficient to drive the circadian activity rhythms in mice [31]. Ageing appears to affect rhythms in some but not all tissues by primarily acting on interactions among circadian oscillators [46], and in hamsters, ageing results in a reduction of entrainment and a breakdown of monophasic rhythms [47]. However, because neither the genetic makeup nor the age of the mole rats used in the present study was known, it was impossible to further explore these possibilities.

4.3. Constant lighting conditions

Monophasic, endogenous locomotor activity rhythms approximating 24 h were expressed under both DD (72.7%) and LL (36.4%), conclusively illustrating that the Mashona mole rat has a circadian rhythm of locomotor activity. Furthermore, the mole rats that displayed an endogenous rhythm and whose rhythms started to drift from the same phase angle to which the animal was entrained under the previous photoperiod (DD=66.7%, LL=100%) undoubtedly support the conclusion that these individuals entrained to the lighting regime and that the entrainment could not have been due to masking [18]. Since not all animals in the sample displayed an endogenous rhythm under DD, and because some individuals switched their preferred time of activity from that of the photoperiod before the constant condition photoperiod, it is not credible to exclude the possibility that the apparent entrainment in these individuals was actually due to masking. It is suggested that the smaller percentage of endogenous rhythms expressed under LL is indicative of constant light negatively affecting the pacemaker of this rodent.

Lovegrove and Muir [14] found a progressive decay of free-running activity rhythms to complete arrhythmia in the solitary Cape mole rat under LL. The same decay of free-running rhythms was observed in some (28.6%) of the Mashona mole rats studied here, although 36.4% of mole rats maintained an endogenous locomotor activity rhythm that approximated 24 h.

Splitting of an activity rhythm (a single daily activity band that dissociates into two components) typically occurs in nocturnal rodents that are subjected to constant light [48]. It has been found to occur in the solitary Cape mole rat [13] and in approximately half of the blind mole rats in the study of Tobler et al. [24], but not in the Mashona mole rats of this study.

Under the constant light conditions, a small variation in τ was observed. This conforms to the predictions made by Pittendrigh and Daan [49], who stated that the variability in τ is smaller in species that display an average τ close to 24 h.

4.4. Masking

The masking response of the two female mole rats indicates that light had the power to override the circadian rhythm control, while the nonmasking response of the males suggests that they were either not able to follow the 3:3 DL cycle or that it negatively affected the circadian pacemaker of these individuals. No conclusive comments can be made based on these masking results, as so little of this behaviour occurred. However, because masking is a behavioural response and not an expression of the endogenous clock, it is hypothesised that the female Mashona mole rat, or possibly only the breeding female, may be susceptible to masking, whereas the males or nonbreeders may not be. Clearly, further studies in this direction are needed.

4.5. Unusual behaviour patterns

It is possible that the two mole rats that did not inverse their locomotor activity rhythm when they were subjected to the LD2–DL switch in photoperiod were not entrained to the first light cycle, but that their apparently entrained rhythm was either a product of a masking response, or an entrainment to some other controlled *zeitgeber*. The same reasons could be responsible for the unusual behaviour of animal 12 throughout the first four photoperiods of this study. Cessation of the unknown cue might be responsible for the light entrainment responses that follow. Also, the

specific shape of the light–pulse phase response curve could account for the failure of the switch in activity after the LD2–DL transition. The reason why the initially dark-repressed animal 12 became light-repressed under the masking photoperiod is unclear.

4.6. Evolutionary relict and progressed sensitivity

As photoreceptors are only found in the mammalian eye [30], the eyes of the African mole rat perform at least one function, namely that of being able to detect light and transfer this information to the pacemaker. In addition to this, some African mole rats also appear to have retained some form of visual ability [5–8,10]. The question is why? It is thought that the blind mole rat eye has maintained or even progressed its photosensitive capacity over time while reducing unutilised metabolically expensive structures [26–29]. It is concluded that the blind mole rat uses photic input, which is received when it occasionally ventures above ground while excavating soil through the mounds, to sustain its thermoregulatory responses and successful reproduction [26,27]. In African mole rats, this does not appear to be the case, as their visual system is markedly different from that of the blind mole rat [5–10]. Especially in the Mashona mole rat, the ability of light detection appears to be of little use as there are potentially individuals that are never exposed to light throughout their life [17]. Therefore, it is concluded that the ability of the Mashona mole rat to perceive and respond to light is likely to be a relict from an ancestral above ground existence. This conclusion is validated by the fact that only blind cave fish that are closely related to their noncave ancestors perceive light [50].

5. Conclusions

There was no evidence of either a diurnal or a nocturnal locomotor activity preference in the Mashona mole rat as a species, but individual mole rats do display entrainment of their locomotor activity to photoperiods, confirming that irradiance differences are perceived by the endogenous pacemaker and utilised as a *zeitgeber*. Evidence for a functional biological clock is provided by the circadian rhythms of locomotor activity under constant dark and constant light photoperiods. Several possible reasons are discussed in an attempt to explain the noticeable interindividual and intraindividual variability in the activity pattern, the diurnal or nocturnal activity preference, and the rate and extent of entrainment that were found.

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