

Circadian rhythms of locomotor activity in captive Emin's mole-rats, *Heliophobius emini*
(Rodentia: Bathyergidae)

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Many African mole-rats (Bathyergidae) exhibit distinct circadian rhythms, despite the fact that they are strictly subterranean and are very infrequently exposed to light. We investigated the circadian system of Emin's mole-rats (*Heliophobius emini*) by exposing 13 individuals to different lighting regimes. The mole-rats were subjected to a standard 12h light:12h dark light cycle, followed by constant darkness (DD) cycle to evaluate entrainment and endogenous rhythms of locomotor activity. After allowing the mole-rats to re-entrain to a 12L:12D light cycle, the light cycle was inverted to examine the duration of re-entrainment following a drastic change in the light cycle. Furthermore, we investigated the effect of different ambient temperatures on the locomotor activity of the mole-rats, and they were thus subjected to a 12D:12L light cycle at 20°C, 25°C and 30°C. Lastly, mole-rats were presented with a long day (16L:8D) and a short day (8L: 16D) light cycle to look at the effect on preferred active time. The animals showed a preference for diurnal activity with nine mole-rats entraining to the light cycle,

and maintaining clear endogenous rhythms throughout DD. Subjects were excessively slow to re-entrain their activity to the inverted light cycle. When subjected to different ambient temperatures, individuals showed marked increases in average activity at 20°C, with the lowest average activity occurring at 30°C. The mole-rats maintain their diurnal preference regardless of day length, condensing their activity during the short day light cycle, while extending their active period during the long day cycle. These results suggest that Emin's mole-rats are able to perceive light and entrain their locomotor activity to light cycles, albeit weakly. In addition, ambient temperature influences the level of overall activity, indicating that these animals may use behavioral thermoregulation to maintain a constant body temperature.

Keywords: Circadian rhythms, Emin's mole-rat, entrainment, *Heliophobius emini*, locomotor activity.

Biological rhythms are essential elements of life and these endogenous rhythms may vary in periodicity from seconds to days (Reuss 1996). Probably the best known and most frequently studied of all biological rhythms is the circadian rhythm (Dibner et al. 2010). Circadian rhythms are expressed over an approximately 24 hour cycle (Reppert and Weaver 2002) and have been found in almost every organism that has been studied to date (Goldman 1999). Circadian rhythms are determined genetically (Vitaterna et al. 2001) and hence are controlled internally. The clock-like mechanism that controls circadian rhythms in mammals is situated in the suprachiasmatic nucleus (SCN) in mammals (Benstaali et al. 2001; Froy 2007).

Circadian rhythms are rarely exactly 24 hours long hence in order to synchronize internal events with the external 24 hour day, the SCN entrains to environmental stimuli, known as *zeitgebers* (Goldman 1999). The most reliable *zeitgeber* in the environment is the daily light and dark cycle (Sharma and Chandrashekar 2005). The SCN controls the output of a variety of rhythms in the physiology and behaviour of organisms, one of which is locomotor activity (Van der Merwe et al. 2014). Although light is the principal environmental stimuli to which the SCN entrains, circadian rhythms may also become entrained to non-photic stimuli thereby masking the underlying photically entrained rhythms (Hastings et al. 1998; Hut et al. 1999; Slotten et al. 2005). The entrainment of the circadian clock to cues such as daily light cycles imparts a survival advantage to organisms by ensuring that the correct physiological processes, such as foraging or mate finding occurs at the correct time of the day (Sharma 2003; Froy 2011).

African mole-rats (family Bathyergidae) are subterranean rodent moles endemic to sub-Saharan Africa. Subterranean mammals by definition spend most of their time underground, mole-rat burrow systems are completely sealed from the surface and the animals are thus generally not exposed to light. Consequently they have evolved an array of specialized morphological and physiological adaptations (Nevo 2007). These include, but are not limited to, an enhanced tactile sense, olfaction and microphthalmic eyes.

Despite the severe reduction in eye size, the eyes of the mole-rats retain the functional capacity to perceive light. Indeed, Němec et al. (2008) showed that morphologically, the eyes of African mole-rats are similar to eyes of typical surface dwelling rodents. However, although the retinohypothalamic tract between the SCN and the retina remains intact, other structures related to vision, such as the visual cortex, has diminished in size when compared to other rodents. In congruence with these findings, previous studies have shown that other members of the family

Bathyergidae are capable of entraining their locomotor activity to light and dark cycles (Oosthuizen et al. 2003; Vasicek et al. 2005; Schöttner et al. 2006; De Vries et al. 2008).

There are 6 genera within the family Bathyergidae, three of which are solitary (*Heliophobius*, *Bathyergus* and *Georychus*) (Jarvis et al. 1994). The species complex of *Heliophobius* mole-rats is the most widely distributed solitary group, occurring from southern Kenya, Tanzania, the Democratic Republic of Congo, Malawi and northern Mozambique (Burda 2001). Like most other mole-rats, Emin's mole-rats (*Heliophobius emini*) are completely subterranean and spend most of their lives underground in burrow systems. Similar to all solitary mole-rat species, Emin's mole-rat and the silvery mole-rat breed seasonally (Šumbera et al. 2003, Ngalameno pers. comm.). Solitary species are highly xenophobic towards other individuals outside of the breeding season, only allowing other animals into their burrows during the breeding season. An accurate mechanism for time keeping is therefore essential to indicate the breeding season.

The natural habitat of the Emin's mole-rat is largely dominated by woodland. The annual precipitation is 966 mm (range 600-1200), with two rainy seasons, a period of heavy rainfall occurring from March to May, and a milder rainfall period from October to December (MRSeP 1997). The annual temperature of the region is relatively mild, with average temperatures of 24.6°C (range 18°C-30°C). Tanzania is on the equator, therefore the annual changes in photoperiod are not dramatic.

Although no information is available on the activity patterns of Emin's mole-rats in their natural environment, the closely related silvery mole-rat (*Heliophobius argenteocinereus*) was found to exhibit activity at any time of the day, although activity tended to be associated with fluctuations in tunnel temperatures (Škliba et al. 2007). Based on this observation, we expected

to find that the Emin's mole-rats would not exhibit robust entrainment of their locomotor activity to light cycles, especially when presented with constant daily temperatures. Hence we aimed to investigate the locomotor activity patterns of Emin's mole-rats under different lighting and temperature regimes in the laboratory. Consequently, we evaluated the ability of the Emin's mole-rat to entrain its locomotor activity to a light cycle and determine its preferred activity time. We also assessed the presence of an endogenous rhythm in the absence of external stimuli and the time to re-entrain the activity rhythm following a drastic change in the light cycle. Subsequently, the effects of ambient temperature and day length on the activity patterns of the Emin's mole-rat were explored.

MATERIAL AND METHODS

Animal capture and housing.—A total of 13 Emin's mole-rats (5 males and 8 females, mean body weight 88.66 ± 26.97 g) were collected from farmlands in the Morogoro region of southern Tanzania. Emin's mole-rat is a member of the cryptic *Heliophobius* genus (Faulkes et al. 2011), that is endemic to Tanzania. The study site is classified as lowland and river valleys agro-ecological zone with highly fertile soils supporting variety of crops include bananas, maize, cassava, cocoyams and sweet potatoes (MRSeP 1997). Species assignment was initially based on proximity of our study site to the type locality and subsequently confirmed through cytochrome *b* (*cyt b*) gene sequence analysis that confirmed the *H. emini* (clade 3) species designation (Faulkes et al. 2011).

Clade and species assignment was determined by *cyt b* gene amplification and nucleotide sequencing of 10 randomly selected individuals (5 males and 5 females), using previously

described primers and thermal cycling conditions (Bastos et al. 2011). Full-length gene sequences submitted to Genbank under accession numbers KX060579-KX060580, were complemented with homologous data from two prior studies (Faulkes et al. 2004, 2011) and the best-fit model of sequence evolution identified was used when inferring a maximum likelihood phylogeny in Mega5 (Tamura et al. 2011).

Animals were captured using modified Hickman live traps baited with sweet potato. The traps were placed into open sections of the burrow system and checked periodically for captured animals. Animals were air freighted to the Republic of South Africa under permit from DAFF and Gauteng Department of Nature Conservation (Permit No: CPB6-2386 and 13/1/1/30/201411000171).

Once the animals were in South Africa they were housed at the University of Pretoria in light (330 lx at floor level) and temperature (according to the experimental condition) controlled rooms. Temperatures in the animal rooms varied with about 0.5°C on either side of the set temperature over the course of the day. Animals were housed individually in plastic crates (58 x 38 x 36 cm) lined with wood shavings. Tissue paper was provided as nesting material and an empty ice cream container cut open on one side that could be used as shelter. The mole-rats were fed *ad libitum* on sweet potato at random times of the day to prevent them from entraining to a feeding schedule. The digestibility and nutritional value of sweet potatoes was previously found to provide animals with adequate nutrition (Bennett and Jarvis 1995). Old food was removed at feeding times when new food was added. The crates were cleaned before the start of each new light cycle. The research procedures followed the ASM guidelines (Sikes 2016) and the capture of the mole-rats and experimental procedures were cleared by the Animal Use and Care committee at the University of Pretoria (EC062-14).

Experimental Setup. —In order to capture the movement of the animals, each cage was fitted with a passive infrared captor [Quest PIR internal passive infrared detector; Elite Security Products (ESP), Electronic Lines, London, UK]. The sensors were placed in such a manner as to detect movement over the entire surface of the cage floor. For the sensors to detect movement, displacement of the animal is required, it does not detect small movements such as grooming. Animals rarely rear up, and when they do, it is against the side of the cage when they are already moving around. The program Vital View was used to capture the activity counts once every minute on a computer system located outside the activity room (Vital View™, Minimitter Co. Inc., Sunriver, OR, USA, <http://www.minimitter.com>).

Light Cycles. —The experiment consisted of nine different light cycles, presented in the following order LD1, DD, LD2, DL25°C, DL30°C, DL20°C, LD3, 8L16D and 16L8D. All of the listed light cycles took place for 29 or more days each, with a few days in-between the different light cycles to allow for the cleaning of cages and for the mole-rats to settle after the disturbance. Emin's mole-rats are induced ovulators, and since animals were housed individually, females did not exhibit hormonal cycles that could have influenced activity patterns (J. Katandukila pers. obs.). The mole-rats were firstly subjected to an LD1 cycle at an ambient temperature (T_a) of 25°C (12L:12D, 09:00-21:00 light, 29 days) in order to assess entrainment of locomotor activity. The LD1 cycle was followed by a constant darkness cycle (DD, $T_a = 25^\circ\text{C}$ 33 days) to detect endogenous free-running rhythms of locomotor activity of the mole-rats.

The animals were then returned to the 12L:12D light cycle (LD2, 09:00-21:00 light, $T_a = 25^\circ\text{C}$, 35 days) in order to allow the mole-rats to re-entrain their activity before exposing them to a new set of light cycles. Subsequently, animals were exposed to an inversed light cycle (DL25, 12D:12L, 09:00-21:00 dark, $T_a = 25^\circ\text{C}$; 47 days). The aim of this inverse cycle was to investigate

the duration of the re-entrainment of the activity following a drastic change in the light-dark cycle.

The inverted cycle was then retained for the following two light cycles, to investigate the possible effects of temperature variations on the locomotor activity of the animals. An inverted cycle at 30°C (DL30) was applied (12D:12L, 09:00-21:00 dark, 32 days), where after the animals were subjected to an ambient temperature of 20°C (DL20; 12D:12L, 09:00-21:00 dark, 33 days).

The animals were then allowed to re-entrain to a normal LD cycle (LD3; 12L:12D, 09:00-21:00 light, $T_a = 25^\circ\text{C}$, 48 days) at 25°C. Once animals were readjusted they were exposed to two additional light cycles, a short day cycle (8L:16D, 11:00-19:00 light, $T_a = 25^\circ\text{C}$, 33 days) and a long day cycle (16L:8D, 07:00-23:00 light, $T_a = 25^\circ\text{C}$, 31 days).

Data Analysis. —The captured data were used to create double-plotted actograms using ActiView Biological Rhythm Analysis 1.2 software (Minimitter Co. Inc., Sunriver, OR, USA, <http://www.minimitter.com>) to provide a graphic representation of the activity patterns of each animal for each light and temperature cycle. Clocklab (ClockLab™; Actimetrics, Evanston, Illinois, USA) was used to determine the total activity counts as well as counts for light and dark periods for each individual animal. The period length (*tau*) of the endogenous rhythm during the constant darkness cycle (DD) was calculated using the Chi-square periodogram function in the rhythm analysis software, Clocklab. The period length of each individual animal was inserted in the program and subjective day and night activity counts were calculated for each animal with its own period length during DD.

Statistical comparisons were performed with IBM SPSS version 23 (SPSS Inc., Chicago, Illinois, USA). Data were not normally distributed and consequently we used a generalized linear

mixed model (GLMM) with light cycle, light phase and gender as main factors in the model. All two and three way interactions were investigated and least significant difference (LSD) was employed for post hoc comparisons. Tau lengths between males and females were compared with a Mann-Whitney U test. Significance levels were maintained at 0.05.

RESULTS

Entrainment of activity rhythms

The mean activity counts for the light and dark phases of each individual animal is provided for each light cycle as a supplementary table (see Supplementary data S1). Overall Emin's mole-rats showed a preference towards a diurnal profile of locomotor activity with significantly more activity during the light phase compared to the dark phase (Figure 1). During the LD1 light cycle, mole-rats displayed significantly more activity occurring during the light phase than dark phase ($F = 265.67$, $df_1 = 1$, $df_2 = 33.57$, $P < 0.001$). Females were significantly more active compared to males during LD1 ($F = 61.40$, $df_1 = 1$, $df_2 = 33.57$, $P < 0.001$). Nine animals showed clear diurnal entrainment to the LD1 light cycle with all activity occurring during the light phase of the cycle (Figure 2a), whereas the remaining four animals exhibited largely diurnal activity, but also a proportion of their activity occurring during the dark phase of LD1 (Figure 2b). One individual did not entrain to any light cycle and remained arrhythmic for the entire duration of the experiment.

Figure 1: Mean activity counts (\pm SE) of male and female Emin's mole rats (*Heliophobius emini*) for the light (white bars) and dark phases (black bars) of each light cycle they were exposed to.

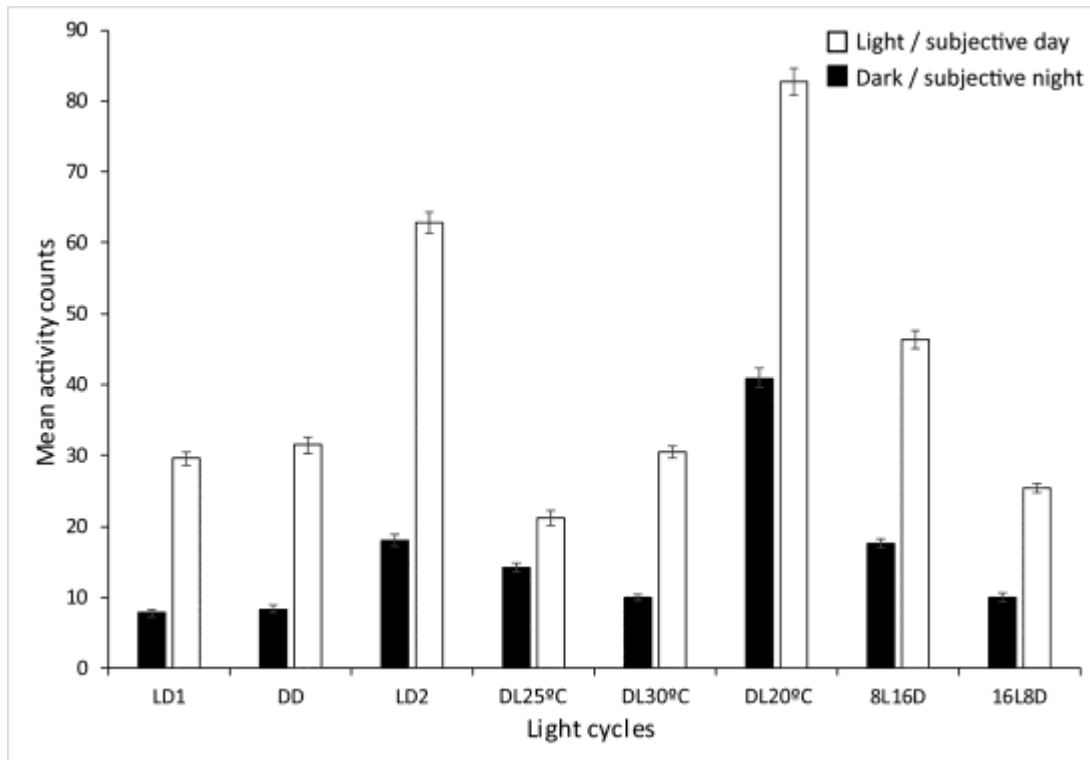
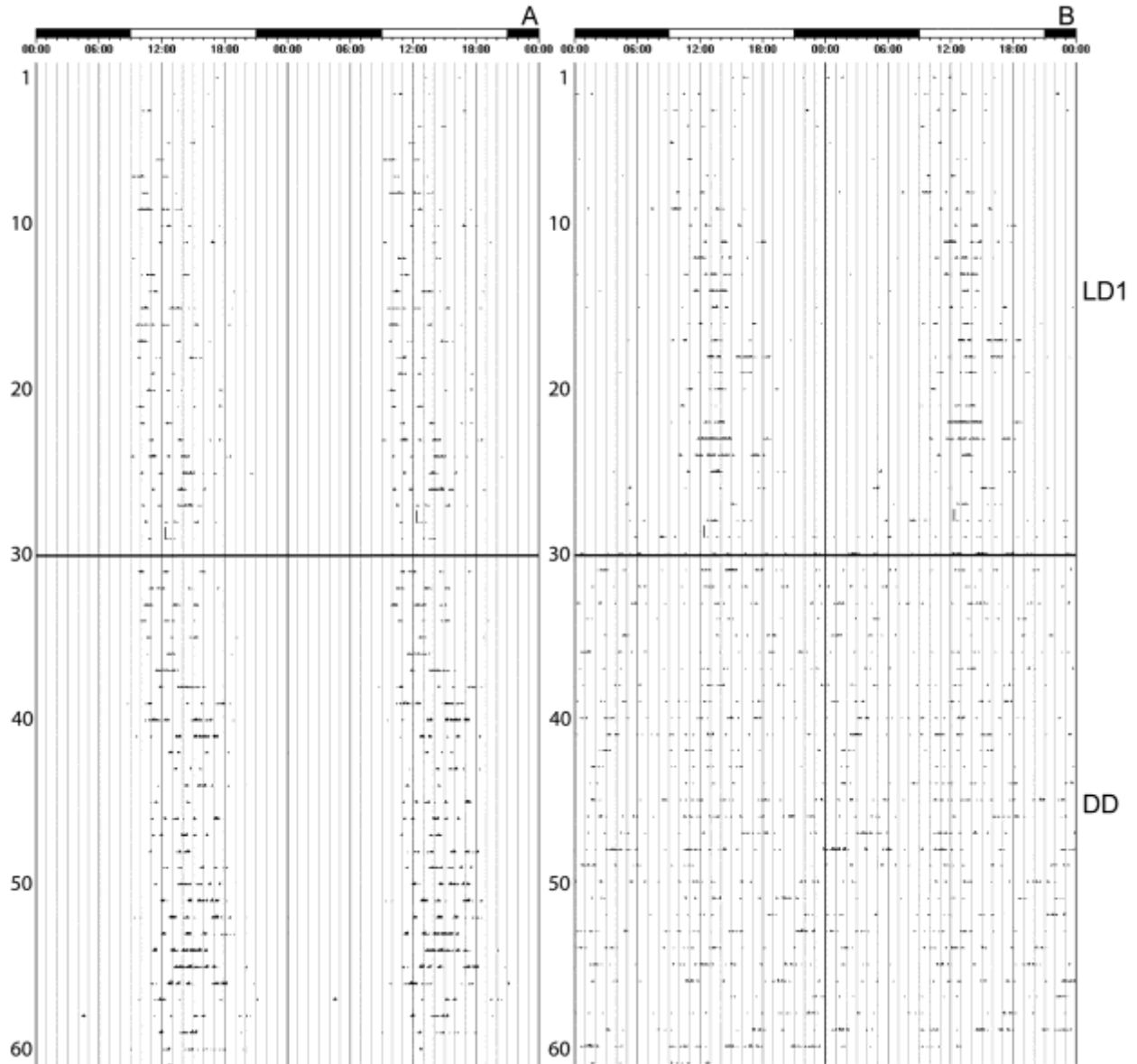


Figure 2: Representative actograms of the activity of two *Heliophobius* mole-rats during the LD1 and DD light cycles. (A) shows an individual with clear entrainment and (B) shows an example of an animal that exhibits entrainment during the LD1 cycle but becomes arrhythmic in DD.



Endogenous rhythms

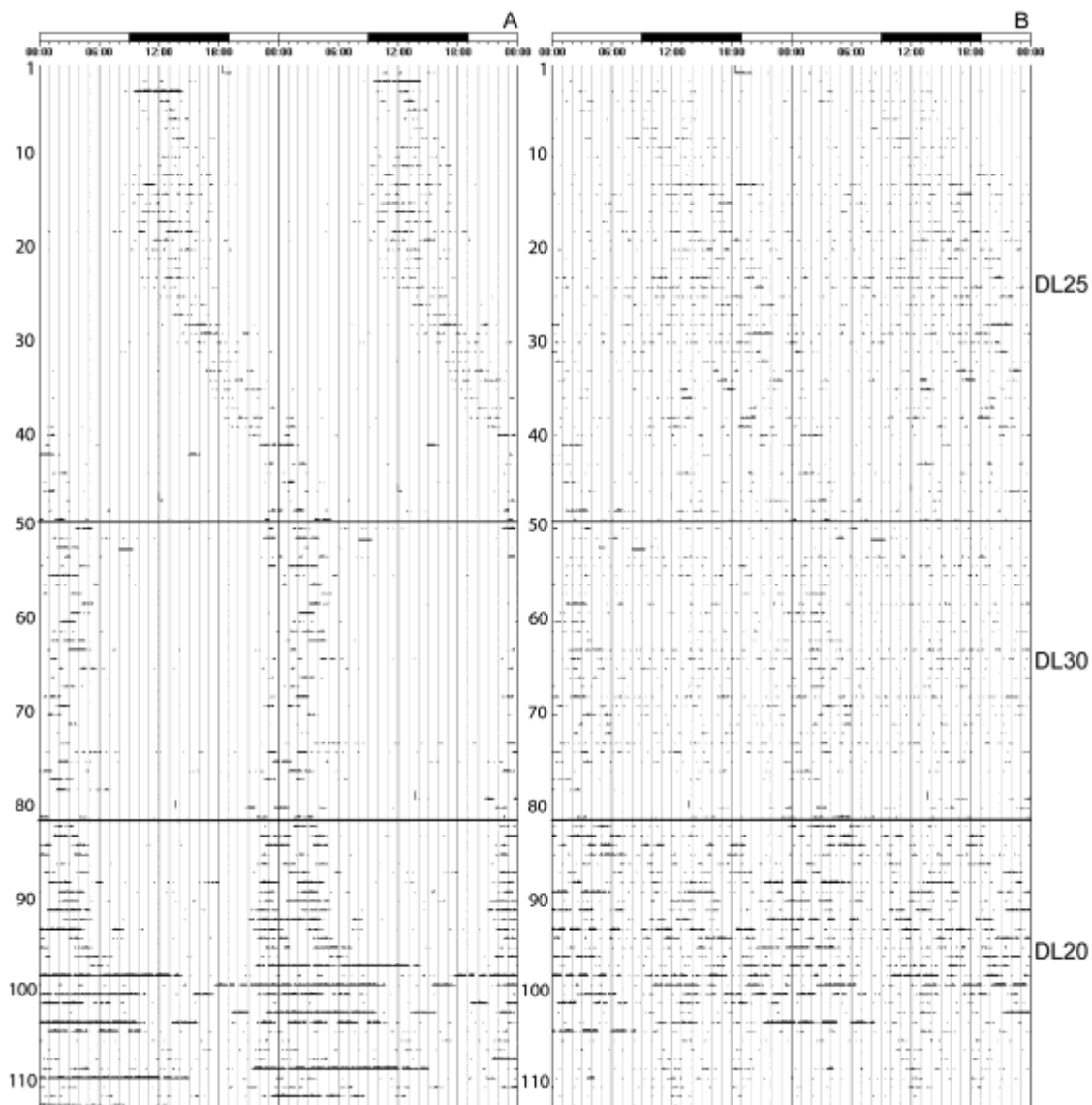
When the animals were subjected to constant darkness (DD) two broadly grouped outcomes were observed. Firstly, some animals ($n = 9$) showed free-running periods very close to 24h and very little difference could be seen between the activity profiles of LD1 and DD (Figure 2a). Secondly, the remaining animals ($n = 4$) showed ill-defined or arrhythmic activity patterns (Figure 2b). The individuals that became arrhythmic were the same animals with more irregular rhythms during the LD1 light cycle. The average activity counts for the DD cycle were comparable with those from LD1 with females once again showing higher level of activity than males. Periodograms could be calculated for all but one animal, which had no clear entrainment. The average length of the free running endogenous rhythms that were calculated for Emin's mole-rats was 23.93 ± 0.14 hours. The tau lengths of males and females were not significantly different (Mann-Whitney test; $Z=-0.488$, $P = 0.639$).

Inverted light cycle

Mole-rats were allowed to re-entrain their activity to an LD cycle (LD2) before they were exposed to an inverted light cycle. Following the inversion of the light cycle seven animals showed clear shifts in their activity patterns towards the light phase of the light cycle. These animals began to shift their activity towards the new light phase only after 18 days or more (24.4 ± 6.11 days) after the switch of the light cycle and required a further 12-30 days (18.9 ± 6.15) to complete their shift to a diurnal pattern (Figure 3a). The animals were considered to be re-entrained to the new light cycle when the onsets of their activity coincided with the onset of the light phase of the new light phase. However, there were four individuals that became arrhythmic for the entire DL25 cycle. The remaining three animals had irregular activity patterns and made no attempt to shift the majority of their activity back towards the light phase of DL25 (Figure

3b). Males showed significantly higher activity counts than females during the DL25 light cycle ($F_{1,34} = 4.82$, $P = 0.028$).

Figure 3: Representative actograms of *Heliophobius* mole-rats showing (A) clearly shifting in its activity pattern after the inversion of the light cycle from LD2 to DL25. The level of the activity remains relatively similar throughout DL25 and DL30 but increases dramatically both in duration and frequency during DL20. (B) shows an animal that became arrhythmic after the inversion of the light cycle and remain so for DL30 and DL20, however the activity increase during DL20 can still be seen clearly.



Locomotor activity at different ambient temperatures

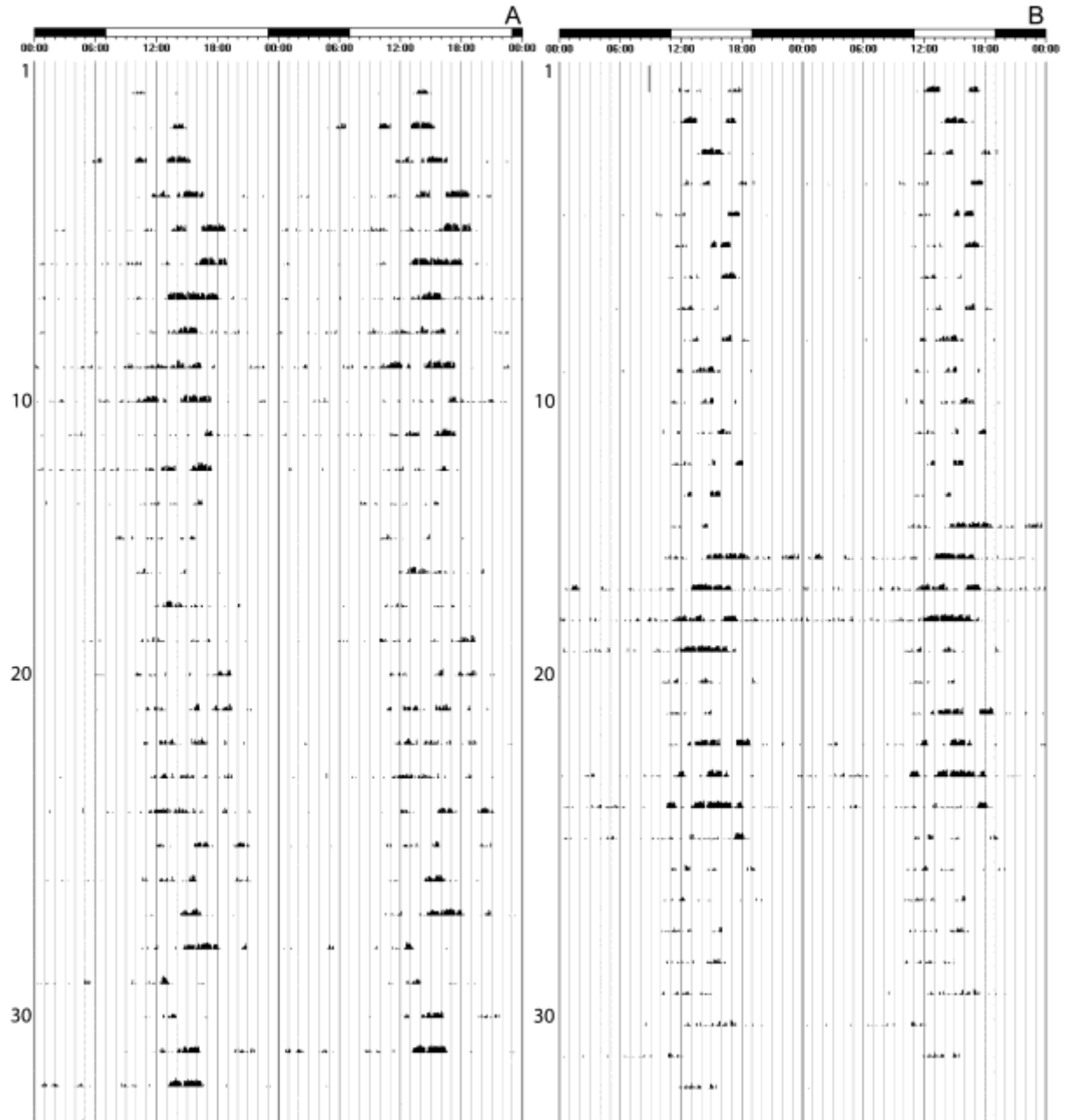
Locomotor activity was compared at three ambient temperatures, data from the LD2 light cycle was used for the 25°C since most animals did not reach a stable activity pattern during the DL25 cycle after the phase shift. A comparison between the three ambient temperatures (20°C, 25°C and 30°C) yielded highly significant differences in the mean activity counts (Figure 1) between all three cycles ($F_{1,30} = 223.74$, $P < 0.001$) with the lowest level of activity at 30°C and the highest activity at 20°C. There was, however, no significant difference between the activity levels of males and females at 30°C ($F_{1,34} = 0.00$, $P = 0.983$), 25°C ($F_{1,34} = 2.46$, $P = 0.117$) or 20°C ($F_{1,34} = 0.984$, $P = 0.321$).

During the DL30 light cycle eight animals displayed clear diurnal rhythms (Figure 3a) whereas the remaining five animals were relatively arrhythmic (Figure 3b). Throughout the DL20 light cycle, in addition to increased overall activity levels, activity bouts also increased in duration (Figure 1). The bouts of activity extended into the dark phase of the light cycle and as a result increased the average nocturnal activity in the DL20 light cycle (Figure 3a). Lastly the prevalence of arrhythmia appeared to increase during the DL20 cycle as seven animals showed irregular entrainment and only six mole-rats showing clearly distinguishable activity rhythms.

Locomotor activity with different day lengths

The activity of the animals during the 8L16D and 16L8D light cycles showed significant preference towards the light phase ($F_{1,28} = 491.42$, $P < 0.001$) showing once again that the species is predominantly diurnal. The 8L16D cycle had much higher average activity counts in both the light and dark phases when compared to the 16L8D light cycle (Figure 1). A comparison between LD1, 8L16D and 16L8D showed a significant difference between all three

Figure 4: Representative actograms of a *Heliophobius* mole-rat (A) on a long day cycle showing slightly extended activity during a long day cycle, (B) and on a short day cycle the animal maintains a preference for diurnal activity, and the onset and offset of activity remain well within the light phase of the cycle



of the light cycles ($F_{2,28} = 163.01$, $P < 0.001$). Both 8L16D and 16L8D showed significant differences between the activity of males and females ($F_{1,28} = 14.72$, $P < 0.001$), ($F_{1,28} = 50.15$, $P < 0.001$) respectively. Figure 4 shows that animals also restricted their activity to the light phases of 8L16D and 16L8D, regardless of the length of the light phase with very little activity taking place during the night phases. Throughout both light cycles 8 animals showed clear diurnal entrainment, whereas the remaining 5 showed unclear entrainment patterns.

DISCUSSION

The timing of activity is widely accepted to carry an adaptive advantage that are species specific, whether it be to synchronize their internal metabolism or to time life events such as migrations (Sharma 2003). Contrary to expectations, Emin's mole-rats exhibited predominately diurnal patterns of locomotor activity when presented with a 12L:12D light cycle. Other mole-rat species in the family Bathyergidae (*Fukomys* spp, *Cryptomys* spp and *Georychus capensis*) that were subjected to similar experiments were found to be mainly nocturnal (Oosthuizen et al. 2003; Vasicek et al. 2005; Schöttner et al. 2006; De Vries et al. 2008; Oosthuizen & Bennett 2015). *Fukomys* and *Cryptomys* are social genera, whereas *Heliophobius* and *Georychus* are solitary (Šumbera et al. 2007). It has been shown in other rodent families that social species are more likely to be diurnal than solitary species (Ebensperger and Blumstein 2006) as social species share the predatory avoidance tasks among the group members and therefore can afford to be active during day time hours where they can more easily be predated upon (Ebensperger and Wallem 2002). Interestingly, a field study on the closely related silvery mole-rat (*H. argenteocinereus*) revealed rhythmicity in their daily activity patterns although not necessarily diurnal. Daily unimodal and bimodal activity peaks were observed at variable times of the day,

although activity was significantly lower during the hottest and coldest times of the day (Šklíba et al. 2007). These results may suggest that in nature, temperature is a more significant and relevant modulator of locomotor activity in these mole-rats. Indeed, considering that *Heliophobius* mole-rats are subterranean rodents that rarely, if ever, comes above ground (Burda 2001), entraining their activity to a specific light phase would be of little use. In the current study, the proportion of animals that showed rhythmicity in their activity ranged from 50-69%. This is a much lower proportion compared to surface dwelling animals, where almost all animals entrain to light (Schöttner et al 2011). Therefore, it may be concluded that the ability to respond to different light stimuli is likely an evolutionary relict from the ancestral strategy of living aboveground (Vasicek et al. 2005).

During the DD cycle of the experiment the light stimulus was removed and nine mole-rats expressed distinct innate endogenous rhythms, while the remaining four animals did not show robust endogenous rhythmicity. Those individuals that displayed clear endogenous rhythms showed very little drift under the DD light cycle indicating that they have periods (τ) close to 24hrs, similar to results found in other *Fukomys* and *Cryptomys* species (Schöttner et al. 2006; De Vries et al. 2008). *Heliophobius* shows the capacity to maintain an endogenous rhythm, in an environment which remains constant for most times of the year with the exception of small fluctuations in temperature in their burrow systems (Šumbera et al. 2004). Indeed *Heliophobius* has been shown to alter locomotor activity patterns in response to such temperature fluctuations (Šklíba et al. 2007).

Emin's mole-rats displayed exceptionally slow response times to the dramatic change in their lighting regimen, with most animals taking an average of 16 days to initiate a shift in their activity patterns. Moreover, the time to be fully re-entrained to the new inverted light cycle was

also extremely long. A high prevalence of arrhythmia was also observed with almost half the animals losing their rhythms completely and those that retained their rhythms shifted the activity very gradually throughout the DL25 cycle. In response to inversed light cycles, bathyergid species show a large amount of variation in the time to re-entrain their activity to the new light cycles. For example, many Mashona mole-rat (*Fukomys darlingi*) individuals became arrhythmic or showed no re-entrainment at all (Vasicek et al. 2005), whereas individuals of the Lesotho mole-rat (*Cryptomys hottentotus* subspecies) showed a rapid reaction to an inverted light cycle with nearly all the animals re-entraining their rhythms in about 9 days (Schöttner et al. 2006). Prolonged re-entrainment periods have also been found in other subterranean rodents that occur in the same geographical area as the Emin's mole-rat; the root rat (*Tachyoryctes splendens*) showed a re-entrainment period of approximately 18 days (Katandukila et al. 2013). It has been suggested that the level of activity an animal is allowed to maintain will affect the speed of its re-entrainment to a light cycle (Castillo et al. 2011). Castillo and colleagues found that by restricting the wheel running activity of mice in a laboratory setting they could delay the period of re-entrainment to light stimulus (Castillo et al. 2011). Emin's mole-rats display overall lower levels of activity when compared to other bathyergid rodents that have been investigated (Oosthuizen et al. 2003; Oosthuizen & Bennett 2015; Schöttner et al. 2006; Vasicek et al. 2005) and as such may be prolonging their re-entrainment due to lack of activity. The variability in the responses observed in Emin's mole-rats may perhaps be attributed to a weak coupling between the pacemaker and the expressed rhythms (Oosthuizen et al. 2003).

It is a well-known that the ambient temperature to which an animal is exposed, whether it be in a laboratory environment or in the natural environment can affect its levels of activity (Gaskill et al. 2009). Emin's mole-rats displayed the lowest level of activity at an ambient

temperature of 30°C whereas the highest level of activity was expressed at an ambient temperature of 20°C. This finding is congruent with another study that has examined the activity patterns of Damaraland mole-rats (*Fukomys damarensis*) under the same temperature regimes (Oosthuizen and Bennett 2015). Oosthuizen and Bennett (2015) found the highest activity under 25°C and the lowest under 30°C. The decrease of activity at 20°C was attributed to the fact that Damaraland mole-rats use social huddling to thermoregulate. Emin's mole-rats are largely homoeothermic and can maintain a constant body temperature of around 35°C over a large range of ambient temperatures (10-32°C), moreover its lower limit of the thermal neutral zone is 28°C therefore 30°C ambient temperature falls within its thermoneutral zone (TNZ) (McNab 1966). Animals are known to prefer ambient temperatures that overlap their TNZ for inactivity (Gaskill et al. 2009), thus it is not surprising that Emin's mole-rats would express the lowest activity counts at 30°C. Decreasing locomotor activity at higher ambient temperatures may have adaptive significance since it will reduce the need to down regulate body temperature through expensive processes such as evaporative water loss. However, when the ambient temperature dropped to 20°C, Emin's mole-rats have to engage in behavioral thermoregulation by increasing its activity as it does not have the advantage of huddling at lower temperatures.

Emin's mole-rats maintained a diurnal preference of activity throughout the short day cycle (8L16D) and the long day cycle (16L8D). During the short day light cycle Emin's mole-rats restricted their activity solely to the light phase of the cycle. The activity appeared to be condensed while having a higher average activity count during the light phase when compared to LD1. The mole-rats did expand their diurnal activity during the 16L8D light cycle but they did not use the full 16 hours with onset and off set of activity always occurring after the onset of the light phase and before the onset of the dark phase. The results obtained from Emin's mole-rats is

congruent with that found in the root rat, *Tachyoryctes splendens*, another equatorial subterranean rodent (Katandukila et al. 2013). However, it should be kept in mind that given that the natural habitat of the Emin's mole-rat is equatorial, these animals would not encounter the drastic changes in photoperiod used in the current experiments, in nature.

The results indicate that the Emin's mole-rat has the ability to distinguish between light and dark and is capable of entraining its locomotor activity to light cycles, even though there is large individual variation. Most animals exhibited endogenous rhythms close to 24 h under constant darkness. Re-entrainment time following an inversion in the light cycle was delayed and exceptionally long, potentially due to overall low activity levels in Emin's mole-rats. Overall these results suggest that the circadian clock of the Emin's mole-rat is less sensitive to light compared to several rodent species that live aboveground. As expected, animals were the least active at an ambient temperature of 30 °C, which overlaps with their TNZ, while the highest level of activity was present at 20 °C, implying behavioral thermoregulation may be necessary to maintain a constant body temperature. The mole-rats maintain their diurnal preference regardless of day length, and are sensitive enough to light stimulus to adjust their activity levels according to the light phase lengths. The functional significance of photic entrainment of the circadian system of subterranean mammals may not be immediately apparent, this may be a relic of a time when vision played a more prominent role in their daily lives. The importance of time keeping in subterranean mammals may not be critical for their day to day lives, but is essential for solitary species that are very frequently seasonal breeders.

Indeed, our study has some limitations. The age of the animals may be a potential factor responsible for the variation exhibited in this study. Age has been found to cause significant changes in locomotor activity patterns (Takahashi et al. 2012). Ages of the animals in our study

were not known. This is further complicated by the lack of published information on the species, including the maximum age of the animals. Mole-rats in the family Bathyergidae reach considerably longer lifespans than is predicted for their body size (Buffenstein and Jarvis 2002, Dammann et al 2011). The closely related silvery mole-rat has been found to live for at least 9 years (Rodrigues and Šumbera 2015), thus Emin's mole-rats is predicted to reach similar ages. The majority of our animals were small in size on capture and were probably subadult. The humidity in the laboratory was approximately 40-50%, significantly lower than that exhibited in mole-rat burrows in the wild. The humidity of silvery mole-rat burrows (*Heliophobius argenteocinereus*; Šumbera et al. 2004) ranges between 69-91%. Humid conditions in the burrow systems may increase thermal stress (Okamoto-Mizuno and Mizuno 2012) and influence the activity levels of individuals, a hypothesis that could be tested with radio-telemetry and behavioral observation in the field. However, the primary aim of this study was to elucidate the potential role of light on the activity of this species and it can be conclusively seen that Emin's mole-rats can both respond and entrain their locomotor activity to the light / dark cycle.

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SUPPLEMENTARY DATA

Supplementary Data S1. Activity counts (\pm SE) for each individual animal for both the dark and light phases of each light cycle the animals were subjected to.

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