Differential locomotor activity responses to day-time light intensity in juvenile and adult solitary Cape mole-rats, *Georychus capensis* (Rodentia: Bathyergidae).

S. Braunstein[1], NC Bennett[1,2], C Voigt,[1,2] MK Oosthuizen[1,2]

[1]Department of Zoology and Entomology, University of Pretoria, Pretoria, 0002, South Africa

[2]Mammal Research Institute, University of Pretoria, Pretoria, 0002, South Africa

Corresponding author: M K Oosthuizen moosthuizen@zoology.up.ac.za

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Abstract

The Cape mole-rat (*Georychus capensis*) is a solitary, strictly subterranean rodent that is responsive to light and entrains to photic cues despite having a reduced visual system. Circadian entrainment is maintained throughout life, but age can alter the amplitude of the response and re-entrainment time. Mole-rats are long-lived for their size which raises questions regarding the robustness of their circadian rhythms and how impacts their locomotor activity rhythms. The locomotor activity rhythms of juvenile and adult Cape mole-rats were investigated. They were exposed to pre-experimental and post-experimental control cycles under fluorescent lights, six 12 h light:12 h dark cycles of decreasing intensities and a constant dark cycle (DD). All animals exhibited more activity during the dark phases of all light regimes. Juveniles were more active than adults and displayed more variable activity during both the light and dark phases. Adults exhibited relatively stable levels of activity under all experimental conditions, whereas juvenile activity decreased as the light intensity was reduced. The amplitude of Cape mole-rat rhythms was consistently low, but similar across light regimes and between adults and juveniles. Cape mole-rats have functional circadian systems, are primarily nocturnal and respond differentially to light intensity depending on their age. Light intensity does not affect the locomotor activity responses of Cape mole-rats in a predictable manner, and could indicate more complex interactions with light wavelengths. The circadian systems of juveniles appear to be more sensitive than those of adults, although the mechanism of the light response remains unclear.

Keywords: juvenile; adult; Cape mole-rat; light intensity; circadian rhythm; locomotor activity

Introduction

Organisms from all taxonomic groups display biological rhythms as adaptations to changing environments (Costa and Kyriacou 2021; Raible et al. 2017). Most mammals are adapted to specific temporal niches and they display specialised features that allow them to optimise their survival in their specific niche. Mammals can be categorised as either diurnal, nocturnal or crepuscular (Dijk and Archer 2009). However, some species have polyphasic activity rhythms which enable them to switch between diurnal or nocturnal behaviour based on their environment (Oster et al. 2002). The hormone melatonin is critical for the regulation and organisation of sleep/wake rhythms and the physiological interpretation of its secretion differentiates the circadian rhythms of diurnal and nocturnal species. In nocturnal species, melatonin stimulates activity, and prolonged exposure to light can reduce activity and disturb their circadian rhythmicity, whereas the opposite is true for diurnal animals (Bilu and Kronfeld-Schor 2013; Hart et al. 2004). In addition to photic cues and non-photic cues, factors such as age and sex may also affect rhythmicity in animals (Carrier et al. 2017; Shoji and Miyakawa 2019; Siwak et al. 2002; Zee et al. 1992). These factors can change the rhythms in terms of intensity and integrity.

Ageing has been associated with sleep cycle disruptions (Zee et al. 1992). While juvenile animals are developing, they have a heightened sensitivity to light and are more responsive to day-length changes in their environment (Catlow and Kirstein 2016; Morita et al. 2021; Zee et al. 1992). Age studies on hamsters suggest that circadian entrainment to light is maintained throughout an animal's life, however, age alters the re-entrainment time as well as the circadian response (Mailloux et al. 1999; Zee et al. 1992). For example, in most rodent species, juvenile animals have a greater locomotor response than their adult counterparts during periods when they are awake, especially in familiar environments (Batis et al. 2010; Catlow and Kirstein 2016; Siwak et al. 2002). Sex-related differences in circadian regulation are due to the different hormones between the sexes (Carrier et al. 2017; Krizo and Mintz 2015). These differences are usually species-specific and interact with age-related effects (Krizo and Mintz 2015).

The most important circadian rhythm is the sleep-wake cycle (Çalıyurt 2017). Thus, changes in light exposure can promote a temporal shift (Khulman et al. 2018). As light conditions remain relatively stable in underground environments, subterranean mammals, such as mole-rats, have adapted to the dark by possessing reduced visual systems and atrophied eyes (Lovegrove and Papenfus 1995; Nemec et al. 2008; Oosthuizen et al. 2010, Oosthuizen and Bennett 2022; Rado et al 1992). African mole-rats, of the family

Bathyergidae, are rodents that inhabit numerous biomes in sub-Saharan Africa (Bennett and Faulkes 2000). Mole-rats rarely surface above ground and live in near-complete darkness (Lovegrove and Papenfus 1995; Oosthuizen et al. 2005). The Cape mole-rat (*Georychus capensis*) occurs in the southwestern part of South Africa in the fynbos biome with isolated populations in the north-eastern regions of Mpumalanga, and KwaZulu Natal (Visser et al. 2017). This species is nocturnal, solitary, and highly aggressive (Bennett et al. 2006; Bennett and Jarvis 1988; Oosthuizen et al. 2003). They are characterised by their russet brown fur with distinct white head patches and large external incisors for digging (Bennett et al. 2006). Solitary mole-rats reach reproductive maturity after approximately 18 months and they are estimated to live up to 10 years (Bennett et al. 2006; Dammann et al. 2022).

Despite their regressed visual systems, mole-rats are able to distinguish between light and dark, and their circadian systems are intact (Němec et al. 2008; Oosthuizen et al. 2005, 2010). A developmental study revealed that as the naked mole-rats ages, important structures in the eye, such as the lens, the anterior chamber, and the ciliary body, become malformed over time, further reducing their limited visual acuity (Nikitina et al. 2004). Some of the same structures are malformed in the eyes of adult Middle Eastern mole-rats (Esquiva et al. 2016). This abnormal postnatal eye development has been observed in fossorial mammals including true moles and mole-rats (Carmona et al. 2010; Esquiva et al. 2016). However, it has been documented that Cape mole-rats respond to light in similar ways to non-subterranean mammals, and they have functional circadian rhythms (Lovegrove and Papenfus 1995; Oosthuizen et al. 2003; Oosthuizen et al. 2005; Oosthuizen and Bennett 2022).

Underground environments have limited time-keeping cues other than temperature fluctuations (van Jaarsveld et al. 2019). However, Cape mole-rats have been observed above ground sporadically (Oosthuizen et al. 2010; Peichl 2005). This raises questions regarding the effect of light intensity on their circadian rhythms. Oosthuizen et al. (2003) showed that African mole-rats possess endogenous circadian rhythms under continuous darkness. In addition, solitary mole-rat species have more prominent activity rhythms when compared to social species and most of the animals exhibited nocturnal activity (Bloch et al. 2013; Oosthuizen et al. 2003). The aim of this study was to investigate the robustness of the Cape mole-rat activity rhythms when exposed to different light intensities and assess age-specific differences in their locomotor rhythms. Based on previous studies, it was predicted that the Cape mole-rat will become more active during the day as light intensity decreases due to the dark conditions, their nocturnal nature, and subterranean lifestyle (Lovegrove and Papenfus 1995; Bennett et al. 2006). We predicted that the juvenile mole-rats would be more active than their adult counterparts as this property has been observed in other species (Siwak et al. 2002). This may be due to postnatal development factors or gene expression changes observed in many fossorial species (Esquiva et al. 2016; Nikitina et al. 2004). In addition, the Cape mole-rat has larger eyes compared to other subterranean species of a similar size and thus may be more sensitive to light intensity changes than other mole-rat species (Nemec et al. 2008; Oosthuizen et al. 2003; Peichl et al. 2004; Peichl 2005). However, we hypothesised that the Cape mole-rat may not be able to perceive low levels of light and would express a free-running circadian rhythm under these conditions. Finally, when no light is present (DD) we predicted that the mole-rats would be more active and have free-running locomotor rhythms of approximately 24 h long.

Materials and Methods

Study Species

Eighteen Cape mole-rats (11 adults and 7 juveniles) were used in this experiment. Eleven adult Cape mole-rats were captured in October 2021 in the Darling area, Western Cape, South Africa (33°22’S, 15°25’E), and transported to the mole-rat research facility in the Department of Zoology and Entomology at the University of Pretoria. Seven juveniles were born in the mole-rat facility at the University of Pretoria and were about three months old at the commencement of the experiment. Animals were individually housed in plastic crates measuring 58cm x 38cm x 36cm. All mole-rats were provided with wood shavings for bedding, a yoghurt tub as a nest and a plastic tube for enrichment. The temperature in the room was maintained at ±25°C and the light cycle was maintained on a 12L:12D photoperiod for the duration of the experiment. The mole-rats were fed daily, and their diet consisted of chopped sweet potatoes and apples with occasional other fruits and vegetables provided. They were fed at a different time each day to prevent entrainment to feeding. Animals were subjected to a series of light conditions involving exposure to different light intensities. At the end of each light intensity cycle, the animal housing containers were cleaned, and the wood shavings were replaced. During the constant dark cycle (DD), feeding and cleaning of containers were done using a red light to limit disruption of the experiment. Since adult animals were wild caught, their exact ages were unknown, however according to their body masses, sizes and body conditions, they were all mature, but not old. All juvenile animals were born in December 2021, roughly three months before the start of the first control cycle. The juveniles were separated from their mothers and littermates prior to the experiment. The experimental procedures were cleared by the Animal Ethical Committee of the University of Pretoria (Number NAS157/2020).

Data Recording and Collection

An infrared motion detector (Quest PIR internal passive infrared detector, Elite Security Products [ESP], Electronic Lines, London, UK) was attached to the top of each container to detect any activity across the floor area. The data were collected at minute intervals by the programme VitalView (VitalViewTM, Minimitter Co., Sunriver, OR, USA) on a computer outside the experimental room.

Experimental Protocol

The mole-rats were exposed to eight light cycles which consisted of a pre-experimental control cycle (Control 1), six distinct light cycles, and a post-experimental control cycle (Control 2). For the duration of the light cycles, animals were exposed to a 12L:12D light cycle where the lights were switched off at 18:00 and switched on again at 06:00 with no intermediate intensities. Each cycle was four weeks long. The first 2 weeks of each cycle served as a habituation period and the data from the following 14 days were analysed. During the control periods, the mole-rats were exposed to fluorescent laboratory lights (measured at below 100 lux at container floor level). LED strip-lights (Inversiones Mesta S.A.C., Lima, PE) were secured above the containers to provide varying light intensities for the experimental cycles. The lights were dimmed using an LED dimmer (LED Dimmed 12-24V 8A 96-192W, Communica, Pretoria, ZA). The order of intensities for the cycles is as follows: 500 lux, 300 lux, 100 lux, 10 lux, 1 lux, and constant darkness (DD). The spectral profile of lighting at each intensity is provided in Supplementary Material Figure S1. All light intensities were measured using a hand-held lux meter (Major Tech, Johannesburg, South Africa). Light intensity was measured at floor level from the centre of the crate.

Statistical Analysis

Data were downloaded from the laboratory computer and prepared for analysis using Microsoft Excel (Version 16.53, 2021). Double-plotted actograms of the mole-rat activity over the course of the cycles were constructed using ActiView (ActiViewTM, Minimitter Co., Sunriver, OR, USA). Data were tested for normality and homogeneity, and as the data were not normal or homogeneous, non-parametric statistical tests were conducted using SPSS

Statistics 27.0 (SPSS Inc., Chicago, IL, USA). Data were summed to obtain a value for the light phase per day, and a value for the dark phase per day. The means of these 12 hour bins were analysed using a generalised linear mixed model, using age (juvenile or adult), light phase (light or dark), and cycle (Control 1, 500 Lux, 300 Lux, 100 Lux, 10 Lux, 1 Lux, DD, and Control 2) as fixed factors with a gamma distribution and an identity link. Day was used as the repeated measure. We attempted to calculate the onset and offset of activity, phase angle, and the alpha (duration of active time), however the mole-rats maintained a low level of activity during the day (non-active time), which made it difficult to determine precise on- and offsets of activity, and also hindered the determination of the alpha (duration of active time). Where possible, the amplitude of the activity rhythms was obtained for each animal per cycle using ClockLab. Amplitude values were compared using Kruskal-Wallis and Mann-Whitney U non-parametric tests. Tau values (individual day length) for the DD cycle were calculated using the periodogram feature in Clocklab (ClockLab, Actimetrics., Wilmette, IL, USA). Statistical significance was maintained at $p<0.05$.

Results

Locomotor activity with decreasing light intensities

The locomotor activity of the solitary Cape mole-rat was significantly affected by light intensity ($F_{7,2438} = 18.432$, $p < 0.001$). Overall activity levels during the control cycles before and after the experimental cycles were comparable. Animals were equally active during the first control cycle and the 500 Lux cycle. Thereafter, activity showed a decrease during the 300 and 100 Lux cycles, a further decrease to 10 Lux. The overall activity showed incremental increases during the 1 Lux cycle, DD (constant darkness) cycle and the second control cycle. Overall, large intra-individual variation was apparent (Table S1). The mean amplitude of the mole-rat rhythms per cycle do not differ over the eight experimental cycles ($H_7 = 6.804$, $p = 0.450$). The amplitudes of the individual rhythms of the mole-rats, were relatively low, and also did not vary significantly over the different cycles ($P > 0.05$ for all animals).

Juvenile vs adult locomotor activity

Juvenile Cape mole-rats were overall more active than their adult counterparts ($F_{1,2438} = 42.959$, $p < 0.001$). Juvenile Cape mole-rats were significantly more active than the adults under all cycles except the 300 Lux ($p = 0.236$), 100 lux ($p = 0.574$) and 1 Lux cycles ($p = 0.147$; Figure 1).

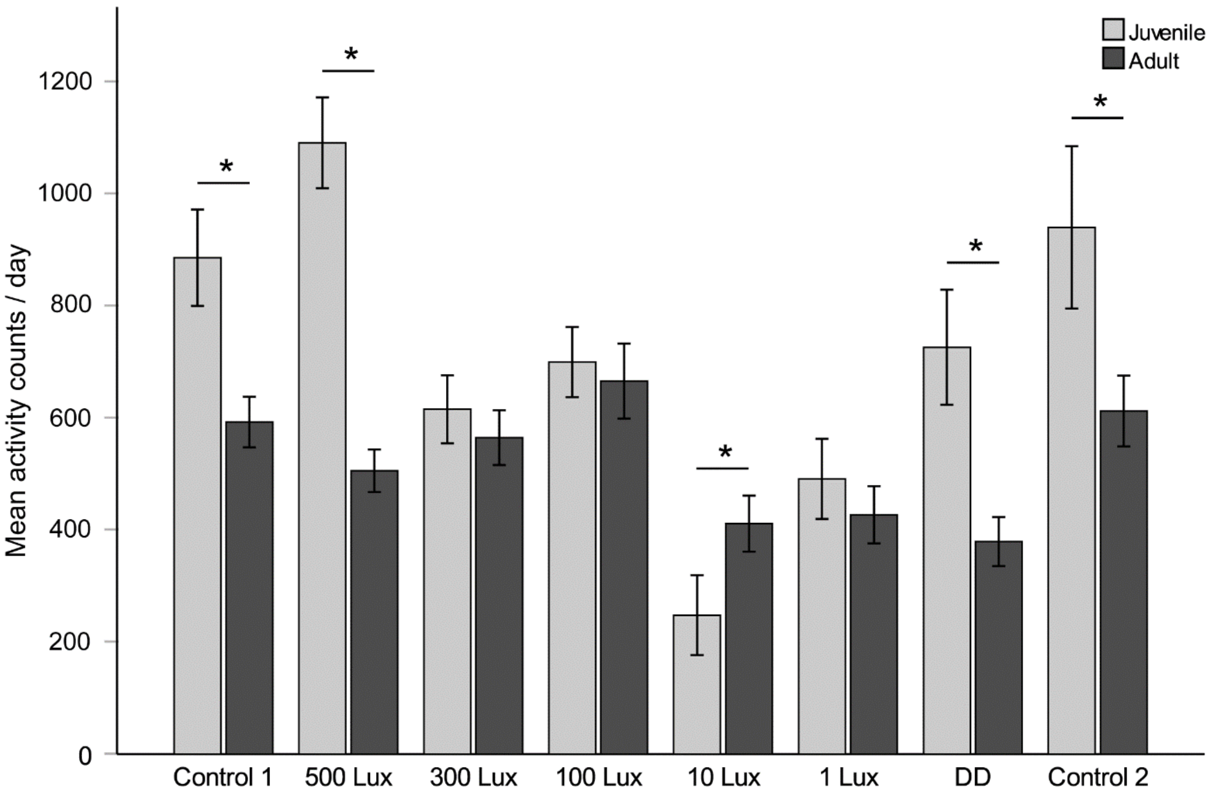

Figure 1. A comparison of the mean overall locomotor activity (± SE) measured per day between juvenile and adult mole-rats for each light cycle.

Locomotor activity of juvenile mole-rats showed an increase from the first control cycle to the 500 Lux cycle and then decreased under the 300 Lux cycle. Activity increased during the 100 Lux cycle before decreasing significantly under the 10 Lux cycle. Thereafter, activity increased during the 1 Lux cycle, the DD cycle, and the Control 2 cycle. The juvenile animals were most active during the 500 Lux cycle and the least active during the 10 Lux cycle. (Figure 1; Table S2).

The overall locomotor activity levels of adult mole-rats showed less variation compared to the juveniles. The activity of adult mole-rats remained constant during the first control cycle, the 500 Lux, 300 Lux and 100 Lux cycles. Thereafter, a significant decrease in activity is observed during the 10 Lux, 1 Lux and DD cycles. Their activity increased significantly again during the second control cycle. The adult mole-rats were most active during the second control cycle and the least active during the DD cycle (Figure 1; Table S3).

All mole-rats exhibited low amplitude rhythms, and the amplitude of juvenile and adult rhythms did not differ (Mann-Whitney U: $Z = -1.533$, $p = 0.125$).

Temporal activity in response to light intensity

Overall, the mole-rats displayed more nocturnal activity than diurnal activity ($F_{1,2438} = 592.648$, $p < 0.001$; Figure 2, 3, see supplementary figures S2-5 for larger, and additional actograms). This was the case for all the light cycles (Figure 4). There was individual variation in the robustness of the locomotor rhythm in response to the light phase. The majority of the mole-rats were nocturnal throughout the LED light cycles, but two juvenile mole-rats exhibited more diurnal activity. In addition, 33% of the mole-rats displayed switches between primarily nocturnal activity to a more diurnal activity and vice versa during the experimental procedures (Figure 5). Locomotor activity levels of the mole-rats changed significantly during the light and dark phases of the different cycles of the experiment (Light: $F_{7,2438} = 16.116$, $p < 0.001$; Dark: $F_{1,2438} = 16.012$, $p < 0.001$). Activity remained relatively constant during the dark phase from the first control cycle to the 100 Lux cycle, whereafter there was a significant decrease in activity to with the decrease in intensity to 10 Lux. Activity increased again during the 1 lux and DD cycles, and thereafter almost doubled when animals were exposed to the second control cycle (Table S4).

Diurnal locomotor activity was overall much lower than nocturnal activity. During the first control cycle and the 500 Lux cycle, diurnal activity was at a similar level. It decreased from 500 Lux to 300 Lux, slightly increased to 100 Lux, and thereafter decreased steadily to 1 lux. Thereafter, there was an increase in locomotor activity during the subjective day in

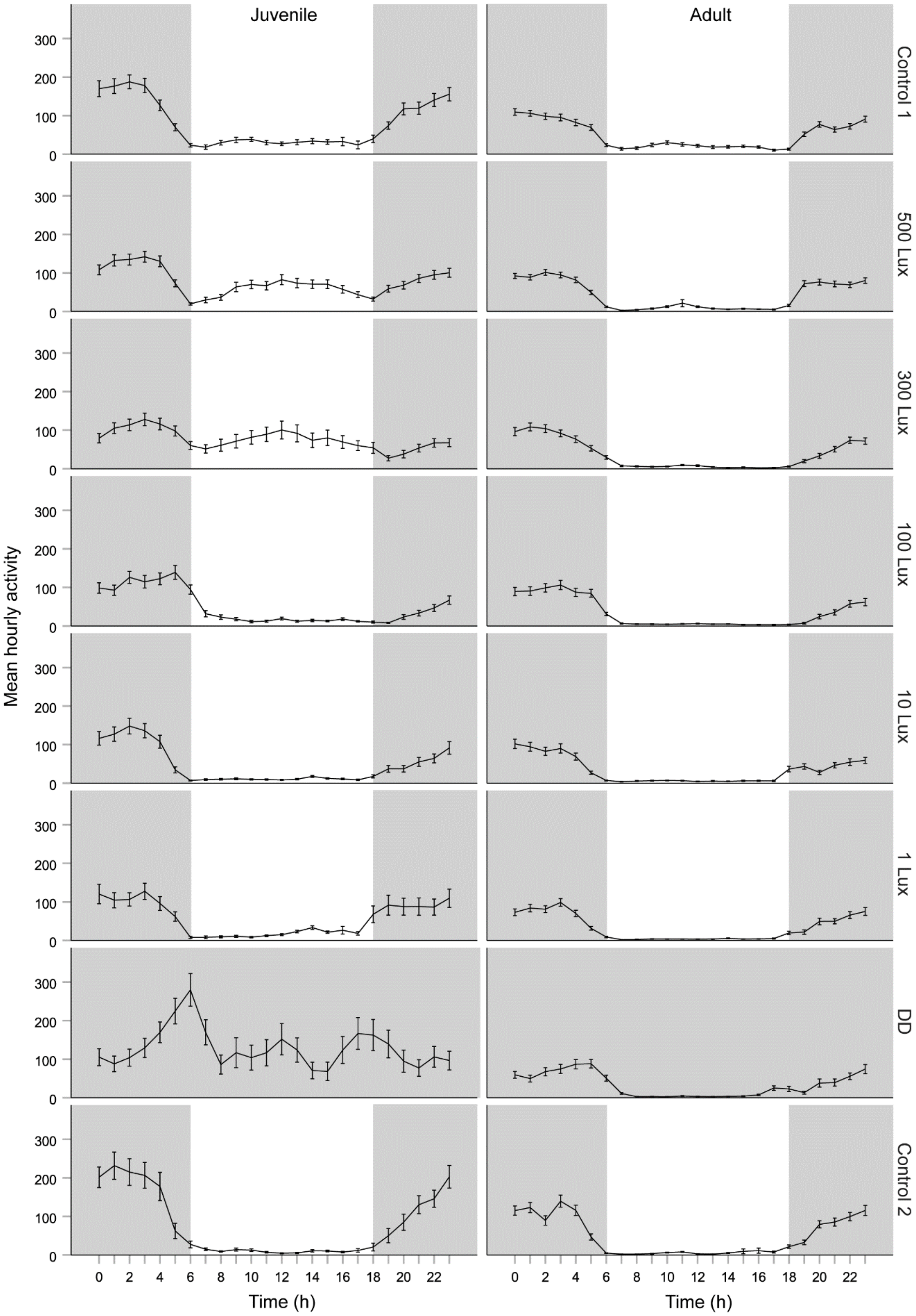

Figure 2. Mean hourly locomotor activity (± SE) of juvenile (left panel) and adult (right panel) Cape mole-rats for each light cycle. Activity is displayed over the 24h of the day, greyed out areas indicate dark periods (light period 06:00-18:00, dark period 18:00-06:00). See supplementary figures S2-5 for larger, and additional actograms.

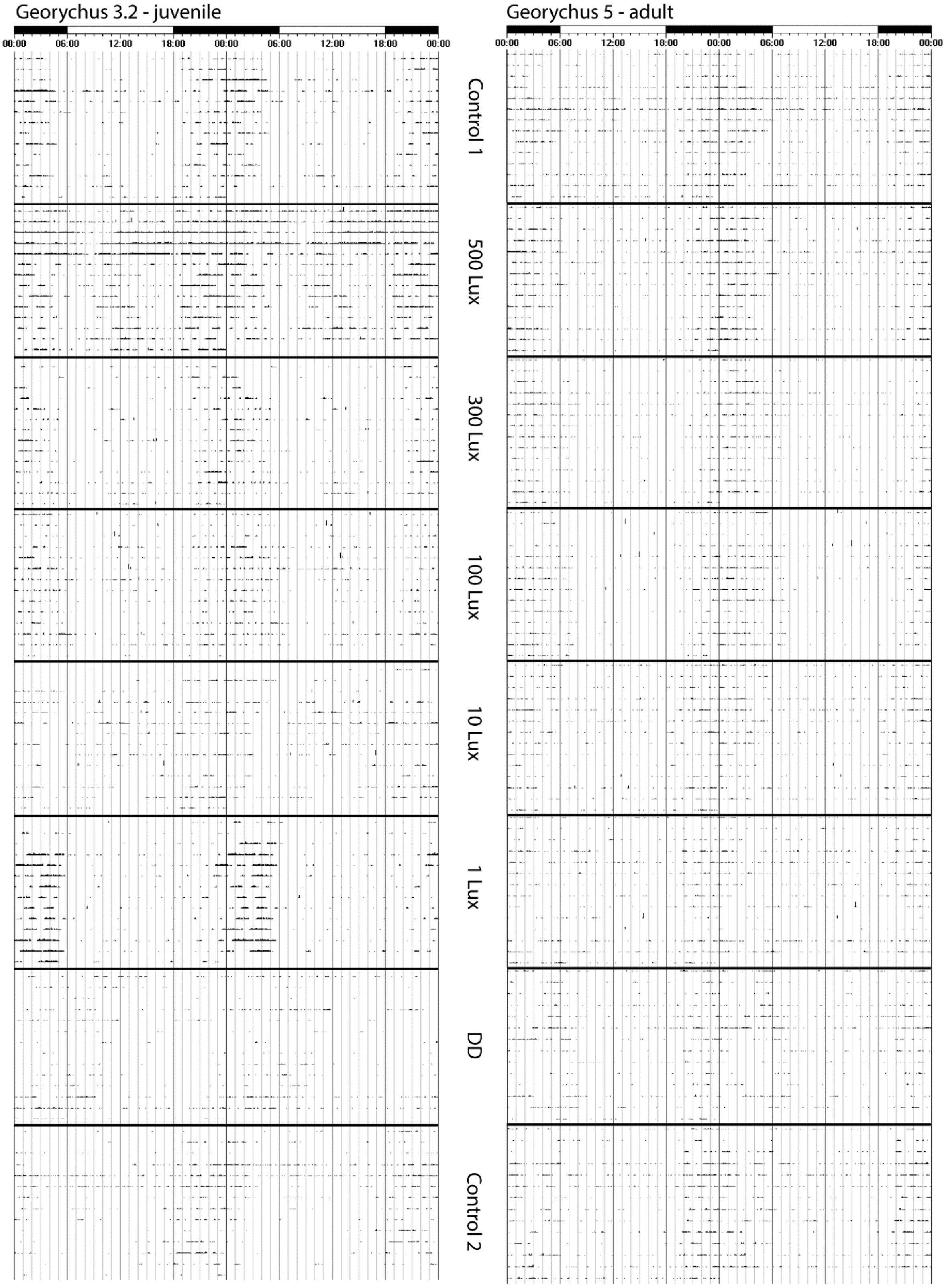

Figure 3. Actograms of two mole-rats for the eight experimental cycles. Left panel is for a juvenile animal, and the right panel is for an adult. Actograms are double plotted, the light and dark bars on top indicate light and dark periods, horizontal lines indicate activity of two days, consecutive lines are consecutive days. Additional actograms are presented in the supplementary material.

DD, and a significant decrease to the second control cycle (Table S4). The increase in activity during the constant dark period is likely related to slight circadian shifts in activity of the mole-rats. The mole-rats displayed activity rhythms with lengths close to 24h when external light cues were removed ($\tau = 23.95$).

The locomotor activity levels of juvenile and adult animals differed during both the light cycles, and the dark or light phases of the respective cycles.

Diurnal activity in the juvenile mole-rats increased from the first control cycle to the 500 Lux cycle and then decreased significantly in the 300 Lux cycle. Their diurnal activity increased slightly, but not significantly during the 100 Lux cycle. Thereafter, the animals were less active under the 10 Lux cycle. Locomotor activity levels increased significantly on the 1 Lux cycle and again during the DD cycle before decreasing sharply in the second control cycle. Juvenile mole-rat diurnal locomotor activity was highest during the 500 Lux cycle and during the DD cycle’s subjective day and the lowest during the 10 Lux cycle. Their nocturnal locomotor activity was at a similar level during the two control periods. It first increased slightly from the Control 1 cycle to the 500 Lux cycle before it decreased significantly in the 300 Lux cycle. Their locomotor activity levels were similar for the 300 Lux, 100 Lux, and decreased again during the10 Lux cycle. During the 1 Lux cycle, the animals exhibited significantly increased activity, it increased again in the DD cycle’s subjective night and significantly more so in the second control cycle. Juvenile nocturnal locomotor activity was lowest during the 10 Lux cycle and peaked during the second control cycle (Figure 4; Table S5).

The total locomotor activity of the adult mole-rats remained at a similar level during the experimental cycles, but the proportion of diurnal and nocturnal activity varied. The diurnal activity levels of adults were significantly higher during the first control cycle compared to the experimental cycles. Their diurnal activity decreased significantly from the first control period to the 500 Lux cycle, increased to the 300 Lux cycle, remained similar for the 100 and 10 Lux cycles, and thereafter decreased in the 1 Lux cycle. Locomotor activity levels increased again during the subjective day of the DD cycle and then decreased significantly from the DD cycle to the second control cycle. Adult diurnal locomotor activity was lowest during the 1 Lux cycle and peaked in the first control cycle. Their nocturnal locomotor activity levels remained relatively stable during the first experimental cycle and 500 Lux, 300 Lux and 100 Lux cycles. There was a sharp decrease in activity during the 10 Lux cycle, whereafter activity levels remained constant during the 1 Lux and DD cycles, and a significant increase is seen in the second control cycle. . Nocturnal activity during the two

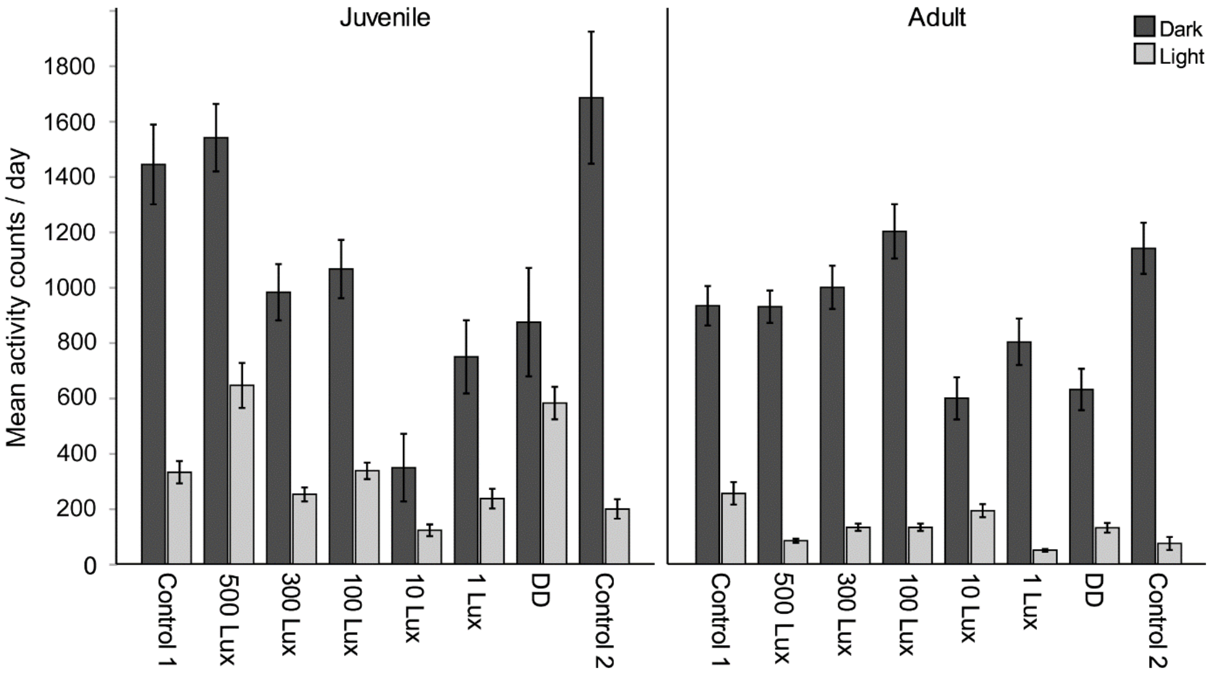

Figure 4. A comparison of the mean day and night locomotor activity (± SE) per day for juvenile and adult mole-rats for each light cycle.

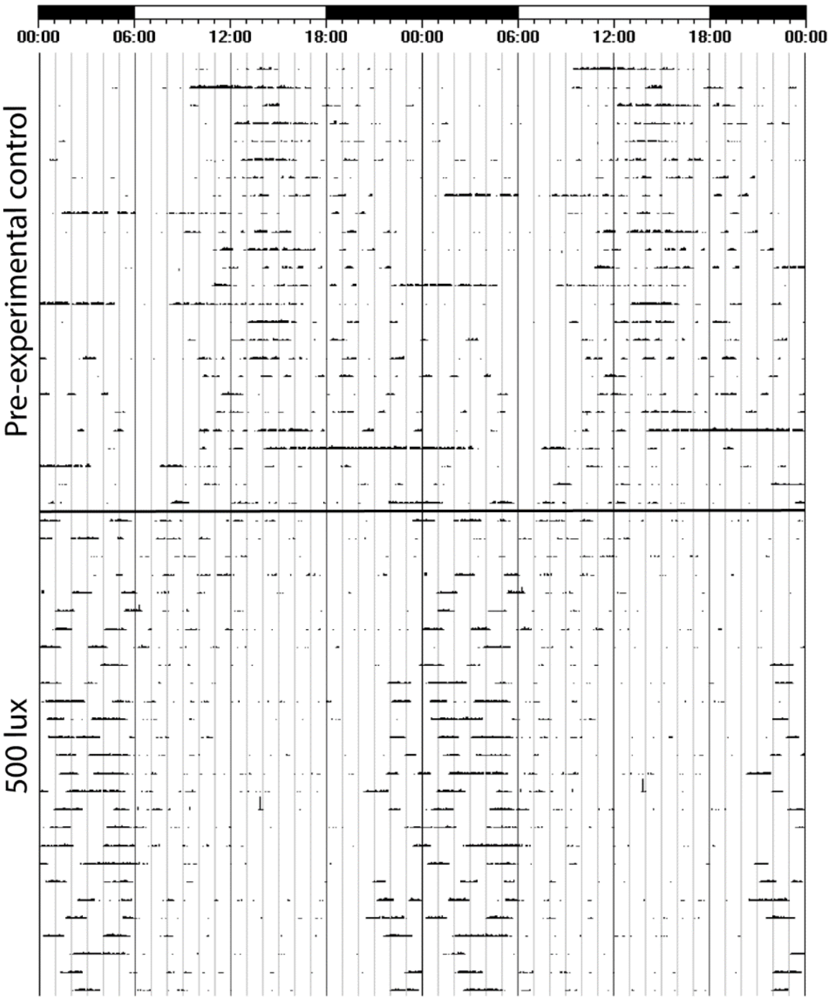

Figure 5. An example of an actogram of a juvenile animal that switched its activity from primarily diurnal to primarily nocturnal. The actogram is double plotted, the light and dark bars on top indicate light and dark periods, horizontal lines indicate activity of two days, consecutive lines are consecutive days. Light is presented during the same time slot for the control and 500 Lux sections.

control cycles did not differ. The adult nocturnal activity was lowest in the 10 Lux cycle and peaked in the 100 Lux cycle (Figure 4; Table S6).

Discussion

Mole-rats are strictly subterranean rodents that are rarely, if ever exposed to light in their natural environment (Lovegrove and Papenfus 1995; Oosthuizen et al. 2010). As such, they have little use for vision and also show morphological adaptations of the eye to their habitat such as reduced eye size, photoreceptors and optic nerve size compared to similar sized surface-dwelling rodents (Bennett and Faulkes 2000; Němec et al. 2008; Nikitina et al. 2004; Omlin 1997; Oosthuizen et al. 2005; Peichl 2005). While visual impairment does not affect the central pacemaker of circadian rhythms (Zee et al. 1992), reduced eye structures as well as the muted conditions in their habitat reduce usable environmental cues for circadian entrainment, limits the use of a circadian system (Oosthuizen and Bennett 2022). However, multiple studies have indicated that mole-rats do possess functional circadian systems although they are believed to express less robust and more variable rhythmicity compared to their aboveground counterparts (De Vries et al. 2008; Hart et al. 2004; Lovegrove and Papenfus 1995; Oosthuizen et al. 2003; Riccio and Goldman 2000; Schöttner et al. 2006). Nevertheless, African mole-rat species range widely in body size and eye size, which could have different implications for the circadian system (Němec et al. 2008; Peichl et al. 2004). We investigated the robustness of the circadian system of juvenile and adult Cape mole-rats in response to decreasing light intensities. This study is the first to compare activity levels in young and mature African mole-rats.

All animals in this study exhibited clear, daily rhythmicity. A previous study characterising the activity rhythms of the Cape mole-rat showed similar results (Oosthuizen et al 2003), as did a study on the solitary Middle-Eastern blind mole-rat (Rado et al. 1992). In contrast, only about 70% of solitary Emin's mole-rats (*Heliophobius emini*) showed entrainment to light cycles, and re-entrainment was incredibly slow in this species (Ackermann et al. 2017). Cape mole-rats show more robust locomotor activity rhythms compared to other mole-rat species, and this is also reflected in the sensitivity of the neurons in the SCN to light (Omlin 1997; Oosthuizen et al. 2005, 2010). In their natural habitat as well as under controlled conditions, the solitary Cape mole-rat is primarily nocturnal in its locomotor activity (Bennett and Faulkes 2000; Oosthuizen et al. 2003). As predicted, the majority of the mole-rats in this experiment were also nocturnal. Even under low-light conditions, their diurnal activity never surpassed their nocturnal activity. The locomotor activity of many nocturnal rodents is masked by light (Ackermann et al. 2020; Alagaili et al. 2012, 2014a,b; Hoole et al. 2017; Van der Merwe et al. 2014; Viljoen & Oosthuizen 2023). These animals usually exhibit almost no activity during the day, and very clear activity on- and offsets such that activity starts after lights are off and terminates before it is switched on again. This is not the case in the Cape mole-rats, most of the animals maintains a low level of activity during the day such that on and offsets of activity, the phase angle and the duration of the active time can not be determined easily.

The amplitude of the circadian rhythms of the Cape mole-rats was overall low. A previous study showed that higher light intensities enhanced the circadian amplitude and the robustness of rhythms in diurnal four-striped mice (*Rhabdomys pumilio*) (Bano-Otalora et al. 2021). The brightness of the light did not affect the circadian amplitude of the Cape mole-rats in our experiment, it remained relatively similar over a range of light intensities spanning from complete darkness to 500 Lux.

In addition, as with other mole-rat species, Cape mole-rats showed individual differences in entrainment (Oosthuizen et al. 2003). In this study, two individuals exhibited diurnal rhythms of locomotor activity, and several animals switched between nocturnal and diurnal activity at some point during the experimental cycles. When subjected to constant conditions (DD), 66% of the mole-rats maintained an endogenous rhythm with a free run period of approximately 24 h. Our results indicate that although the Cape mole-rat can clearly perceive light and entrain to it, its rhythms are rather flexible and not particularly robust compared to surface dwelling rodents.

As predicted, the juvenile mole-rats were more active throughout the 24-hour period than the adults. This is a common feature in mammals and has been documented in several other rodent species such as mice, rats, and Syrian hamsters (Batis et al. 2010; Labyak et al. 1998; Shoji and Miyakawa 2019; Siwak et al. 2002; Spear and Brake 1983). Throughout the experimental cycles, the juvenile mole-rats expressed larger changes in activity between cycles compared to their adult counterparts, however the amplitude of the circadian rhythms did not differ between juveniles and adults. A reduction in the amplitude of body temperature and melatonin rhythms has been observed in older rats and humans (Hofman and Swaab 2006; Nakamura et al. 2011; Skene et al. 2003). This could indicate that the adult animals in our experiment were not aged, or the circadian rhythms of the mole-rats are not robust enough to detect these types of changes.

Since our animals were always fed during the light hours, increased juvenile activity could be result from anticipation towards food. Young rats have been shown to became more active leading up to a scheduled mealtime, whereas the adult rats were less active before the mealtimes (Shibata et al. 1994).

The adults had clearly defined periods of inactivity, usually during the day, and became more active later in the evening after the lights had been switched off. This mirrors results found in older mice in which their endogenous active periods were closer to the entraining cue which resulted in a delay of activity and shorter active periods (Valentinuzzi et al. 1997). Alterations in the response of the circadian system to external cues take place during adulthood, resulting from changes in the neural structure of the circadian clock (Labyak et al. 1998). Studies on hamsters showed that it takes longer for older animals to respond to stimuli, and a study on mice revealed that older animals show signs of lower light detection via retinal ganglion cells (Morita et al. 2021; Zee et al. 1992). Stronger external signals may be required for older animals to elicit a more robust circadian response (Labyak et al. 1998).

A major characteristic of circadian rhythms is the ability to adjust behaviour and physiological output in accordance with the signal received by the SCN (Kramer and Birney 2001). The intensity of the ambient illumination affected the amount of activity exhibited by the animals, but not in a linear fashion. In fact, when regarding the entire cohort of animals, the light intensity had a rather variable effect on locomotor activity. Overall, locomotor activity of the mole-rats was higher during the brighter illumination, and lower at light intensities below 100 Lux, although it did not decrease in a linear fashion. Activity increased in both juvenile and adult animals during the constant darkness cycle, indicating that high light intensities did not suppress or mask activity. Activity during the second control cycle was comparable to that of the first, illustrating that the reduction in activity is not as a result of the lengthy experiment. This response was different from that observed in another study on a social mole-rat species, the highveld mole-rat (P. Chanel, unpublished), which showed that locomotor activity increased as the light intensity decreased. Upon further scrutiny, it turned out that the variation in the Cape mole-rat was largely present in the juvenile animals. The adults displayed relatively constant levels of activity throughout the experimental cycles, while the activity of the juvenile animals was much more variable. The activity levels of the adult Cape mole-rats resemble that of the crepuscular four-striped mice (*Rhabdomys pumilio*), which also did not show much change with light intensities ranging from 1 to 330 lux (Van der Merwe et al. 2017). Juvenile Cape mole-rats showed similar variable activity levels compared to nocturnal Namaqua rock mice (*Micaelamys namaquensis*) over the same range of light intensities (Van der Merwe et al. 2017).

Differences in locomotor activity can depend on age and development (Mailloux et al. 1999). It is evident that the age of the mole-rats plays a significant role in circadian entrainment and locomotor activity responses to light. As animals age and develop, they undergo many physiological and behavioural changes (Carrier et al. 2017; Catlow and Kirstein 2016; Mailloux et al. 1999; Shoji and Miyakawa 2019). Animals are born with immature circadian rhythms, which eventually synchronise to their immediate environment (Nikitina et al. 2004). Once they reach adulthood, further changes take place that initiates a decline in circadian function (Zee et al. 1992). Previous rodent studies have shown that circadian rhythmicity declines in expression with age (Biello et al. 2019; Kramer and Birney 2001; Labyak et al. 1998; Morita et al. 2021; Zee et al. 1992). For example, as rats and mice age, their ability to entrain to light conditions decreases (Mailloux et al. 1999; Morita et al. 2021; Valentinuzzi et al. 1997). A further study demonstrated that as Syrian hamsters become older, they display a loss of responsiveness to circadian phase-shifting stimuli such as increased or decreased levels of light (Labyak et al. 1998). Similarly, the adult mole-rats did not alter their activity patterns as much as the juvenile mole-rats did. This may be due to the weakened light sensitivity of the melanopsin-based photoreceptors in the retina and thus decreased light sensitivity of the SCN (Biello et al. 2019; Labyak et al. 1998; Lupi et al. 2012).

While familiarity to an environment does impact age-related activity (Siwak et al. 2002) the varied response of the juvenile animals does not result from habituation to the laboratory conditions, since activity during the control periods before and after the experimental conditions are similar, and activity increased significantly during the DD cycle. The lower activity levels of the adult mole-rats are consistent with previous studies on above-ground rodents (Shoji and Miyakawa 2019; Valentinuzzi et al. 1997).

The LED strip lights have a distinct short wavelength peak (445nm) at higher light intensities, but this peak is significantly reduced at 10 lux and disappears at 1 lux (see supplementary S1). The retinas of mole-rats contain a higher proportion of cones, in particular, cones that are sensitive to short wavelengths (Peichl et al. 2004), and melanopsin (contained in a subset of retinal ganglion cells), the photoreceptor that mediates circadian responses, are also sensitive to the blue range (Mure et al. 2007). The activity of the juvenile mole-rats appears to reduce as short wavelength light information diminishes, but interestingly then increases again once light is completely absent. Most animals retained their rhythmicity in DD, while increasing their activity. In above-ground rodents such as mice, the free-running period for juvenile animals was longer is longer than those of adult animals

(Valentinuzzi et al. 1997) whereas no difference was apparent in adult and juvenile Cape mole-rat rhythm periods. Therefore, further studies would be required to elucidate the sudden increase in activity during DD.

Many above-ground animals are nocturnal, this has evolved for predator avoidance (Julien-Laferriere 1997). Therefore, it is likely that circadian rhythms and light sensitivity in Cape mole-rats are vestigial remnants of an aboveground ancestor (Oosthuizen et al. 2005). Even limited vision may aid mole-rats in predator avoidance or patching up holes in their tunnels (Oosthuizen et al. 2010; Peichl 2005). In addition, similar to other solitary mole-rat species, Cape mole-rats are seasonal breeders (Bennett & Jarvis 1988; Hart et al. 2006; Herbst et al. 2004; Oosthuizen & Bennett 2007, 2009). Therefore, the necessity of producing offspring when enough resources are available may explain why the selective advantage of circadian rhythms has remained intact (Oosthuizen et al. 2010, Oosthuizen & Bennett 2022). It is evident from our results that Cape mole-rats have endogenous circadian rhythms and are capable of entraining to light cues. However, the low amplitude of the rhythms indicate that they are not very robust compared to surface-dwelling animals. Light intensity does not affect locomotor activity of Cape mole-rats in a predictable manner, and variation in activity may result from more complex, unexplored interactions with light wavelengths. Furthermore, this study demonstrates that the age of Cape mole-rats (juvenile vs adult) impacts light sensitivity and the amplitude of SCN responsiveness.

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Disclosure statement

The authors report no conflict of interest.

Data availability

The data that support the findings of this study are available from the corresponding author, MKO, upon reasonable request.

Author contributions

SB – student, experimental setup, performed experiment, analysis, wrote original draft

NCB – co-supervisor, conceptualisation, funding, revision of final manuscript
CV – animal capture, revision of final manuscript
MKO – supervisor, conceptualisation, experimental setup, analysis, writing and revision of final manuscript

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