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Visual capabilities in subterranean rodents

Ph.D. Thesis

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Anotation

This Ph.D. thesis focuses on the visual capabilities of subterranean African mole-rats (family Bathyegidae) and a potential role of their residual vision. We described basic visual capability of light/dark discrimination and capacity to perceive short to medium-wavelength light in the photopic range of intensities. Our behavioural findings revealed severe visual deficits, implying visually guided spatial orientation in mole-rats improbable. Our field study demostrated no light-correlated daily activitivy pattern of mole-rats in the natural habitat. We described very effective propagation of long wavelength light in breached burrows that can be detected by mole-rats for long distances, in contary to short wavelengths with very low propagation. Thus, an unique primary blue light perception, described only in African mole-rats among all mammalian species so far, has most probably no adaptive value and might be only a result of arrested cone development. Mole-rats effectively localized and plugged with soil illuminated sites under laboratory conditions, supporting the hypothesis that low acuity residual vision play an indispensable role in bathyergid anti-predatory behaviour and tunnel maintanance but not in spatial orientation.

Declaration [in Czech]

Prohlašuji, že svojí disertační práci jsem vypracoval samostatně, pouze s využitím pramenů a literatury uvedených v seznamu citované literatury

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své dizertační práce, a to v nezkrácené podobě – v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou - elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

V Českých Budějovicích, srpen 2015

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„The only thing worse than being blind is having sight but no vision“.

Helen Keller

List of papers and author´s contribution

The Thesis is based on the following papers (listed chronologically):

I. **Kott O,** Němec P, Fremlová A, Mazoch V and Šumbera R, (in prep.): Behavioural tests reveal severe visual deficits in the strictly subterranean African mole-rats (Bathyergidae) and efficient vision in the fossorial rodent coruro (*Spalacopus cyanus,* Octodontidae)

Ondřej Kott participated in development of the experimental design, he collected 50% of data, he participated in procession and evaluation of the results, and he wrote first draft of manuscript.

II. **Kott O**, Moritz RE, Šumbera R, Burda H and Němec P, 2014: Light propagation in burrows of subterranean rodents: tunnel system architecture but not photoreceptor sensitivity limits light sensation range. *Journal of Zoology,* 1-9

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Ondřej Kott developed the experimental design of the field measurements and collected all field data, participated in procession and evaluation of the results, he also participated on writing manuscript.

III. Šklíba J, Lövy M, Ema Hrouzková, **Kott O,** Okrouhlík J and Šumbera R, 2014: Social and Environmental Influences on Daily Activity Pattern in Free-Living Subterranean Rodents: The Case of a Eusocial Bathyergid. *Journal Biological Rythms*, 203-2014

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Ondřej Kott participated in data collection and commenting on the first draft of the manuscript.

IV. **Kott O**, Šumbera R, Němec P, 2010: Light Perception in Two Strictly Subterranean Rodents: Life in the Dark or Blue?. *PLoS ONE* 5(7) : e11810.doi:10.1371 /journal.pone.0011810 (IF $_{2010}$ = 3,742)

Ondřej Kott participated in the experimental design, he collected all data, he participated on procession and evaluation of the results and he led writing of manuscript.

Contents

Chapter 1

Introduction and thesis overview

Introduction

Vision

Vision is important sense for most species of vertebrates, including humans receiving vast majority of informations from the outside world using the visual system (Dusenbery 1992). That is most probably the main reason vison has fascinated people from the dawn of civilization (Howard 1996).

If we trace back through the fossil record, it appears that vision originated in the early Cambrian, some 530 million years ago (Land 2002). Based on paleontological evidence eyes are thought to have evolved independently in different organisms at least 40 times and possibly as many as 65 times (Salvini-Plawen and Mayr 1977). Despite the incredible diversity among extant eyes, the laws of physics constrain how light can be collected resulting in only eight known optical systems in animal eyes (Fernald 2004). All eye types share the same molecular strategy of absorbing photons. Across many species there is a notable continuity of the developmental genes that participate in the construction of similar - but not necessarily homologous - eyes (Fernald 2000). Seeing is important for most species and it has probably been a key selective advantage throughout the animal evolution. In some species, vision is the dominant sense while it is less important in others.

Vision in mammals and the environment

Mammals, standing at the top of the vertebrates evolution, have adopted various lifestyles associated with different light conditions and visual challenges.

Most nonprimate mammals possess dichromatic color vision based on two cone types with spectrally different visual pigments: a short-wavelength-sensitive (S-)cone and a longwavelength-sensitive (L-)cone. Vertebrates possess one type of rhodopsin (visual pigment) located in the rods and four various opsin types located in four various cone spectral types. Four groups of vertebrate cone opsins are named according to their rough spectral sensitivity maxima: SWS1 (short-wavelength-sensitive 1, near UV and violet), SWS2 (short-wavelengthsensitive 2, violet and blue), RH2 (middle-wavelength-sensitive, green) a LWS (longwavelength-sensitive, yellow and red). Rods contain opsin RH1, which is closely related to cone opsin RH2, indicating that the rod evolved out of a cone-like photoreceptor. Mammals have lost RH2 and SWS2 opsins and they possess only LWS and SWS1. Therefore, the most prevalent system of color vision in mammals is known as dichromacy (see Dusenbery 1992, Yokoyama 2000, Fernald 2000, 2004, Jacobs and Rowe 2004).

Various deviations from basic dichromatic pattern, basen on short (SWS) and long (LWS) wavelenght sensitive cones in retina, occur in mammals (Peichl 2005). The presence of middle-to-long sensitive cones (MWS), and hence the potential for trichromatic color vision have been demonstrated in four marsupial species (Arrese *et* al. 2002, 2005a,b). Efficient trichromatic color vision re-evolved only in Old World primates and man by a duplication of the LWS opsin gene (reviewed in Jacobs 1993).

Cone monochromacy, the possession of one cone type only, and, thus the absence of conebased color vision, is rare among mammals. For example, some of terrestrial mammals have lost (S-)opsin expression and possess only (L-)cones. The absence of (S-)cones has previously been reported in a few nocturnal mammals, for example, in the family Procyonidae. The nocturnal racoons *Procyon lotor*, *Procyon cancrivorus*, and the nocturnal kinkajou (*Potos flavus*) completely lack (S-)cones and posses only (L-)cones (Jacobs & Deegan 1992). This is also the case for nocturnal primate species, such as the owl monkey (*Aotus trivirgatus*) and the greater bushbaby (*Galago garnetti*) (Wikler & Rakic 1990) and two species of African giant rats (*Cricetomys gambianus, C. emini*) (Peichl & Moutairou 1998). However, the absence of (S-)cones is not exclusively associated with nocturnality. It was also reported in two species of earless seals (*Phoca hispida, P. vitulina*) with diurnal and nocturnal activity (Peichl & Moutairou 1998). The same kind of deficit seems to be the rule among marine mammals. The pinnipeds and the cetaceans have lost their (S-)cones and became (L-)cones monochromats in the evolutionary process of adapting to marine environment (Griebel & Peichl 2003, Peichl 2005). It has been suggested that blue cones were lost in the early ´coastal´ period of cetacean and pinniped evolution since many coastal waters preferentially absorb blue light and constitute a long-wavelength-dominated environment. On the other hand, other aquatic and semi aquatic mammals have retained two spectral cone types ((S-)cones and (L-)cones). In semi aquatic species terrestrial activities probably had some impact on keeping cone dichromacy. This was also reported for the fully aquatic manatee (*Trichechus manatus*) (Anhelt & Kolb 2000) or semi aquatic pygmy hippotamus (*Choreopsis liberiensis*) (Peichl *et* al. 2001).

Modern mammals lost their ability to see ultraviolet in the course of evolution contrary to birds and lower vertebrates (Land 2002). However, in a few mammalian species we can find (S-)cones with maximal sensitivity in the near UV (around 360 nm). This spectral sensitivity shift possess, for example, different degus (*Octodon degus,O*. *bridgesi, O*. *lunatus*) (Chavéz *et al*. 2003), or cururo (*Spalacopus cyanus*) (Peichl *et* al. 2005). In these species it is used as an adaptation to visual demands during certain phases of surface activity. Fresh cururo and degu urine has a high UV reflectance, suggesting that scent marks may be visible to the UV-sensitive cones and could serve in a territorial behavior and spatial orientation (Chavéz *et* al. 2003, Peichl *et* al. 2005). On the other hand, no clear ecological value of UV sensitive cones was found in rat (*Rattus norvegicus*) having primarily a nocturnal activity (Jacobs *et* al. 2001). The same mechanism, but with a different morphological basis, evolved in the nectar feeding flower bat *Glossophaga soricina* of Central and South America. These bats pollinate rainforest plants, having a strong reflection of UV-spectrum light at night. *Glossophaga* lost the functional cones and retained only the rods as photoreceptors catching UV-photons. In these mammals only a single photoreceptor is responsible for the perception of light radiation over the whole wavelength spectrum, from about 310 nm to 600 nm (Winter *et al*. 2003). An unique S-cones domaninace (app. 90%) in retina, thus a pontetial of primary blue light perception, was described in African mole-rats living underground. This trait, without any obvious adaptive value, was described only in this family of subterranean rodents among all mammalian species so far (Cernuda-Cernuda *et* al. 2003, Peichl *et* al. 2004).

Vision underground

Sensory perception plays a crucial role in spatial and temporal orientation, foraging and communication with conspecifics. Like their surface dwelling counterparts, subterranean animals must find and recognize a mate, kin, intruders and danger. The subterranean environment restricts communicative signals and it is also deprived of most spatial and temporal orientation cues available aboveground. Since burrowing is energetically demanding, it is expected that energetically costly sensory organs and brain centres, which are useless underground, should regress (Nevo 1999, Burda 2003). However, about 300 mammalian species have adapted to the stable, low-oxygen and dark underground ecotope (reviewed in Nevo, 1999; Lacey, Patton & Cameron, 2000). Subterranean mammals, which live and forage underground, have small eyes. While some of them such as the marsupial mole (*Notoryctes typhlops*), the blind mole rats (Spalacidae), the golden moles (Chrysochloridae) and some moles (Talpidae) possess minute, subcutaneous eyes with a degenerated optical apparatus and vestigial visual system, the only function of which is to detect ambient light for photoperiod perception, some others, not less adapted to subterranean existence, such as the African mole-rats (Bathyergidae), zokors (Myospalacinae) and some moles (Talpidae) have quantitatively reduced, but structurally normal eyes that have retained the capability of image-forming vision (for review, see Burda, Bruns & Muller, 1990; Němec *et* al., 2007).

African mole-rats (Bathyergidae)

The African mole-rats are one of the most specialized groups of subterranean rodents. They are endemic rodents to Sub-Saharan Africa. Members of genera *Bathyergus, Georychus*, and *Heliophobius* are solitary, whereas other three genera *Heterocephalus, Fukomys* (this genus has been emancipated from genus *Cryptomys*, see Kock *et* al. 2006), and *Cryptomys* are highly social or even eusocial (cf. Patzehauerová *et* al. 2013). They are food specialist on geophytes – plants with subterranean storage organs (Shermann *et* al. 1991, Bennett & Faulkes 2000, Burda 2001). The silvery mole-rat (*Heliophobius argenteocinereus*, Peters 1846) inhabiting Tanzania, Malawi, southern Kenya, southeast D. R. Congo, eastern Zambia, and northern Mozambique, has the largest distribution among bathyergids (Šumbera *et* al. 2007). It possesses long, dense hair of greyish color sometimes with a white head spot (Nowak 2001). Males weigh on the average 190 g, females around 162 g (Šumbera *et* al. 2003a). In general, they live in mesic areas (> 900 mm annual rainfall) and in elevations of up to 2000 m a. s. l., but they can also be found in arid areas (for example in Kenya) (Šumbera *et* al. 2007). The silvery mole-rats live in various types of habitat, but mostly in hard soils of woodlands dominated by *Brachystegia* or *Combretum* trees (miombo woodland) (Šumbera *et al*. 2007). They build long, highly branched burrow systems, and they change its architecture seasonally (Šumbera *et* al. 2003b) and have low population densities (Šumbera *et* al. 2007). The giant-mole rats (*Fukomys mechowii,* Peters 1881) live in northern Zambia, south D. R. Congo, and Angola. They also occur in habitats and soils with annual rainfall of around

1100 mm (Kawalika *et al*. 2007). They possess beige or light brown pelage. Males weigh 250-600g and females 200-355g (Scharff *et* al. 1999). They are highly social, probably eusocial, and they form groups of up to 40 members (Scharff *et* al. 2001). They construct large burrow systems with seasonal architectural changes (Sichilima *et* al. 2008). The Mashoma mole-rat (*Fukomys darlingi*) are herbivorous, socially living subterranean bathyrdids. They live in miombo woodland and shrub habitats (Bennett *et* al. 1994). They form families containing approximately seven animals (5-9) where reproduction is restricted to one breeding pair (Bennett *et* al. 1994). This species occurs in Eastern and Northen Zimbabwe, and it is believed to also lived in Western Mozambique (c. f. Bennett and Faukels, 2000).

Eye morphology and vision in African mole-rats (Bathyergidae)

Because of living in a monotonous dark environment, the African mole-rats were described as fully blind (Eloff 1951, 1958, Burda *et* al. 1990). However, morphological studies in three bathyergid species, *Fukomys anselli*, *F. Mechowii*, and *Heterocephalus glaber*, reported an unexpected conservation of the mole-rats visual apparatus (reviewed in Němec *et* al. 2007, 2008). The mole-rats possess microphthalmic eyes (2 mm diameter) superficially positioned and with normal ocular properties such as eyelids, clear cornea, lens, vitreous and iris with pupilary aperture (Cernuda-Cernuda *et* al. 2003, Peichl *et* al. 2004). Except for the naked mole-rat´s large lens, the lens size in other mole-rat species is surprisingly small in relation to their eye size (Nikitina *et* al. 2004, Peichl *et* al. 2004). This pattern is typical for diurnal mammals, in contrast to animals with nocturnal activity, which have larger lenses to collect light more effectively. In three species of mole-rats, *F. mechowii*, *F. anselli*, and *H. glaber*, rod-dominated retina with unexpectedly high cone proportion (≈10 %, density 8000-15000/mm2) was described. This cone/rod ratio in subterranean mammals is also more similar to diurnal than to nocturnal surface-dwellers. In *F. anselli* nearly all cones express the (S-)opsin in their outer segments. Many of these (S-)cones co-express small amounts of (L-)opsin, but there are only few pure (L-)cones expressing (L-)opsin exclusively. This (S-)opsin dominance and low levels of (L-)opsin were first described across the entire retina (Cernuda-Cernuda *et* al. 2003, Peichl *et* al. 2004).

The optic nerve is thin and macroscopically barely visible in bathyergids. The very low number of optic nerve fibers and hence retinal ganglion cells indicates poor visual resolution (Cernuda-Cernuda *et* al. 2003, Němec *et* al. 2004). In the African mole-rats all subcortical visual centres are cytoarchitectually poorly developed and reduced in size, while degree of reduction differs between nuclei. The lateral geniculate complex and the pretectal nuclei are moderately reduced (Němec *et* al. 2004). Nuclear expression of transcriptional regulatory protein (c-Fos) as a marker of cell activity was examined immunohistochemically in *F. anselli* (Oelschläger *et* al. 2000). The olivary pretectal nucleus, which in mammals is involved in light/dark discrimination, as well as, the dorsal lateral geniculate body as part of the image-forming visual system, were consistently labeled by c-Fos after light stimulation, indicating that vision in *Fukomys anselli* plays more significant role than previously assumed (Oelschläger *et* al. 2000). Another laboratory study reported c-Fos expresion in the suprachiasmatic nucleus which is synchronized according to the phase of the circadian clock in solitary *Georychus capensis* (Oosthuzien *et* al. 2005). On the other hand, the accessory optic system, responsible for mediation of optokinetic response in reaction to a slow motion of large visual stimuli across the retina, is vestigial and incomplete in mole-rats (Němec *et* al. 2004, Crish *et* al. 2006). Also the superficial layers of superior colliculs, a brain structure involved in the detection of, and orientation toward objects in the peripheral visual field, allowing approach or avoidance decisions, are extremely reduced (Němec *et* al. 2004).

These morphological findings indicate that visual signals cannot be effectively processed by the brain structures and thus used in a surface spatial orientation. In contrast, subsystems involved in a photoperiod perception, form and brightness discrimination are anatomically rather well developed, and, therefore, may still play an important role (Němec *et* al. 2004).

Visual capabilities and role of residual vison in African mole-rats

Even though a question of potential visual capabilities in mammals living underground has provoked since the time of ancient Greeks (for example Aristoteles), only very few studies behaviourally dealed with this topic in subterranean rodents before our projects (e.g. Rado *et* al. 1992, Werner *et* al. 2005, Wegner *et* al. 2006). Besides rather anecdotic study (Eloff 1958), the only one behaviourally focused on light perception in African mole-rats (Wegner *et* al. 2006). Thus basic questions, such as: „What visual capabilities do the African mole-rats still possess and why do they retain unreduced energetically costly vision even after their long underground existence?“, or: „Does the primary blue light perception in these subterranean rodents have any adaptive value?“, remained unanswered. Our main goal was to answer them, at least partly.

In this thesis, we described discrimination between light and dark in two species of mole-rats, so this basic visual capability is now known in four species and one hybrid of mole-rats from two genera: *Fukomys anselli, Fukomys kafuensis* and their hybrids (Wegner *et* al. 2006), *Fukomys mechowii* and *Heliophobius argenteocinereus* (**Kott *et* al. 2010, chapter 2**). In *F. mechowii* and *H. argenteocinereus* capacity to perceive short to medium-wavelength light in the photopic range of intensities was described for the first time (**Kott *et* al. 2010, chapter 2**). These behavioural findings of light perception capability are consistent with anatomical evidence (Cernuda-Cernuda *et* al. 2003, Peichl *et* al. 2004). Further, we bring original results on visual capabilities in strictly subterranean African mole-rats (**Kott *et* al. in prep, chapter 5).** No single behavioural test has been conducted to assess image-forming vison in subterranean mammals before our study. On the basis of known neuroanatomy of regressed brain visual centres processing the visual signals (Němec *et* al. 2004, Crish *et* al. 2006), implying minor importance of image-forming vison, we selected three basic spatial visual tasks (VPR - Visual Placing Reflex, VC - Visual Cliff, CC - Cue Card Test) to test if the mole-rats are able to involve their residual vision in their surface orientation or not. We compared visual capabilities of strictly subterranean African mole-rats with fossorial coruro (*Spalacopus cyanus*) and surface dwelling laboratory mouse (*Mus* *musculus*, inbred C57/L/J). The behavioural assays revealed severe visual deficits in all mole-rats species tested (*Heliophobius argenteocinerus, Fukomys mechowii* and *F. darlingi*). In VPR experiment, mole-rats displayed no spontaneous reaction (forepaw extension) when lowered toward a visible surface. In VC experiment, a random choice between the shallow and the deep side of the visual cliff clearly demonstrated inability of mole-rats to perceive depth. On the contrary, coruros and laboratory mice exhibited a clear placing reaction and preferred the shallow side of the visual cliff, imlying functional image-froming vision. The nesting assay (CC), based on orientation towards a visually contrast part of arena, did not yielded conclusive evidence regarding capacity for visually guided spatial orientation in mole-rats. Behaviuoural data gathered in our study (**Kott *et* al. in prep, chapter 5**) demonstrate that vision is seriously compromised in strictly subterranean, congenitally micropthalmic African mole-rats, a finding also consistent with anatomical evidence (for review, see Němec *et* al. 2007).

Our laboratory findings indicate that light/dark perception is the primary visual capability in African mole-rats. That is why we focused more on role of light in mole-rat´s life. Cycle of light is generally tought to be the strongest and the most universal zeitgeber in vertebrates (Reffinetti 2006). In mammals living in a stable environment with lack of external cues that would potentially reset their circadian clocks, we would expect circadian activity rythms to be weak or absent. However, subsystems involved in a photoperiod perception are suprisingly well developed and purposeful in these strictly subterranean rodents (Oelschläger *et* al. 2000, Němec *et* al. 2004) and indeed ability to synchronize their activity with light/dark cycle under laboratory conditions was presented in several species of African mole-rats (e.g. Lovegrove & Papenfus 1995, Oosthuzien *et* al. 2003, Vasicek *et* al. 2005) or blind mole-rat (*Spalax ehrenbergi)* (e.g. Rado *et* al. 1992, Ben-Shlomo *et* al. 1995, Goldman *et* al. 1997). On the contrary, field studies in *Heliophobius argenteocinereus* (Šklíba *et* al. 2007), *Fukomys damarensis* (Lovegrove 1988), *F. mechowii* (Lövy *et* al. 2013) and *F. anselli* (**Šklíba *et* al. 2014, chapter 4**) demonstrated no light-correlated daily activitivy pattern. We presented that during the cold season *Fukomys anselli* had one activity peak (around 2 pm) which was tightly correlated with the temperature measured at depth of foraging burrows. This pattern of activity is probably a result of minimizing the cost of thermoregulation, because this small species (*F. anselli*) has high thermoregulatory requirements to maintain stable body temperature below the lower critical temperature **(Šklíba *et* al. 2014, chapter 4)**.

In the field, we also described light propagation in opened burrow system of mole-rats. We made very first measurements of light intensities in experimentally opened burrows of Ansell´s mole-rat (*Fukomys anselli*) in the natural habitat and compared them with the experimental measurements under laboratory conditions (**Kott *et* al. 2014, chapter 3)**. Light propagations in natural and artificial tunnels were in accord. In both cases, we demostrated an effective propagation of middle and long wavelengths and low of short wavelengths. In the natural habitat, amount of light which penetrates to a damaged burrow system of Ansell´s mole-rat during a day and also a moonlight night suffice to inform an underground dweller about the site of potentially increased predation risk (**Kott *et* al. 2014, chapter 3).** In the same study, we partly unveiled and raised a new discussion about an enigma of S-cones (primarily sensitive to short wavelengths) dominance in retina, described only in African mole-rats among all mammals so far (Cernuda-Cernuda *et* al. 2003, Peichl *et* al. 2004). It seems cone arrangement of mole-rats retina is not primarily suited for vison in burrows (low propagation of short wavelengths). We suggested, a persistence of this obviously non-adaptive feature in mole-rats might be a result of arrested cone development caused by congenital hypothyrodoism. It is known that cones switch expression from the S- to L-opsin in some species during early postnatal development (reviewed in Lukáts *et* al. 2005). The thyroid hormone receptor *β*2 plays a key role in S-opsin repression and L-opsin activation during cone transdifferentiation (Ng *et* al. 2001, Yanagi, Takezawa & Kato 2002). Low levels of thyroid hormones have been reported in the naked mole-rat, *Heterocephalus glaber*, and associated with their subterranean life (Buffenstein *et* al. 2001). Although it is not known whether low thyroid hormone levels also hold in other bathyergid species, an intriguing possibility that S-cones dominance in bathyergid retina is a mere mechanistic consequence of hypothyroidism certainly deserves investigation. This very new hypothesis should be a subject of further laboratory projects.

A general role of light perception and use of still very energetically costly reduced visual system in strictly subterranean, congenitally microphthalmic mole-rats might seem confusing. The available anatomical evidence strongly suggests that their visual systems are neither suited for above-ground spatial orientation nor adapted for low-light vision (cf. Němec *et* al., 2007, 2008; **Kott *et* al. 2010, chapter 2**). Therefore, it has previously been suggested that besides maintaining circadian rhythms the primary function of the residual, low acuity vision is to localize breaches in the burrow systems that let in light and thereby act as an early warning system helping subterranean mammals to avoid predators (Hetling *et* al., 2005; Wegner *et* al. 2006, Němec *et* al. 2007, 2008). Laboratory experiments have indeed demonstrated light-evoked aversive behaviour in three species of African mole-rats (Hetling *et* al., 2005; **Kott *et* al. 2010, chapter 2**) and the same type of behaviour was demonstrated in fossorial pocket gophers (Werner, Nolte & Provenza, 2005).

On the basis of our findings, light perception seems to be the primary visual function of the residual vison in African mole-rats that serves as an simple and efficient mechanism involved in anti-predatory behaviour and tunnel maintanance. Other experiments, in which no appetitive motivation is required, will be needed to assess the capacity for visually guided spatial orientation in the bathyergid mole-rats. As the bathyergid mole-rats are known to be able to swim (Hickman 1983, 1988), the Morris water maze (Morris 1984) seems to be a particularly promising experimental paradigm for future research.

Conclusions

The African mole-rats possess residual, low acuity vision and are able to perceive short to medium-wavelength light. Spatial visual capabilities are reduced and visually guided aboveground orientation seems to be exluded. Light does not work as an evironmental factor of daily activity pattern in natural habitat of mole-rats. Long wavelengths of light propagate in burrows very effectively and can be detected by mole-rats for long distances, in contary to short wavelengths. Thus, an unique blue light perception has most probably no adaptive value and might be only a result of arrested cone development. Our findings support the hypothesis that low acuity residual vision play an indispensable role in bathyergid anti-predatory behaviour and tunnel maintanance but not in spatial orientation.

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Chapter 2

Light Perception in Two Strictly Subterranean Rodents:Life in the Dark or Blue?

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Light Perception in Two Strictly Subterranean Rodents: Life in the Dark or Blue?

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Abstract

Background: The African mole-rats (Bathyergidae, Rodentia) are strictly subterranean, congenitally microphthalmic rodents that are hardly ever exposed to environmental light. Because of the lack of an overt behavioural reaction to light, they have long been considered to be blind. However, recent anatomical studies have suggested retention of basic visual capabilities. In this study, we employed behavioural tests to find out if two mole-rat species are able to discriminate between light and dark, if they are able to discriminate colours and, finally, if the presence of light in burrows provokes plugging behaviour, which is assumed to have a primarily anti-predatory function.

Methodology/Principal Finding: We used a binary choice test to show that the silvery mole-rat *Heliophobius argenteocinereus* and the giant mole-rat *Fukomys mechowii* exhibit a clear photoavoidance response to full-spectrum (''white''), blue and green-yellow light, but no significant reaction to ultraviolet or red light during nest building. The mole-rats thus retain dark/light discrimination capabilities and a capacity to perceive short to medium-wavelength light in the photopic range of intensities. These findings further suggest that the mole-rat S opsin has its absorption maximum in the violet/blue part of the spectrum. The assay did not yield conclusive evidence regarding colour discrimination. To test the putative role of vision in bathyergid anti-predatory behaviour, we examined the reaction of mole-rats to the incidence of light in an artificial burrow system. The presence of light in the burrow effectively induced plugging of the illuminated tunnel.

Conclusion/Significance: Our findings suggest that the photopic vision is conserved and that low acuity residual vision plays an important role in predator avoidance and tunnel maintenance in the African mole-rats.

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Introduction

Sensory perception plays a crucial role in animal spatial and temporal orientation, foraging and communication with conspecifics. Animals have to find and recognize a mate, kin, intruders or danger. Among senses, vision is important for many species and it has probably been a key selective advantage throughout the animal evolution [1,2]. In the context of visual ecology, the subterranean niche is arguably the most extreme sensory environment, being deprived of light and all visual cues available above ground. However, because it provides shelter from predation and climatic fluctuations, about 250 mammalian species have adopted subterranean lifestyle [3–5]. The darkness of the underground ecotope not only relaxes the selection acting on the visual system, but through the metabolic gain yielded by visual system reduction also imposes a selective pressure acting in favor of its regression [3,6]. Indeed, strictly subterranean mammals are congenitally microphthalmic and possess a severely reduced visual system, although the degree of reduction vary substantially among species (for review, see [7,8]).

The African mole-rats (Bathyergidae, Rodentia) are strictly subterranean rodents endemic to sub-Saharan Africa. They inhabit extensive burrow systems isolated from the aboveground environment by mounds of soil and feed almost exclusively on geophytes, i.e., plants with subterranean storage organs [9]. In most species, the above ground activities appear to be restricted to rare events such as dispersal and mate-seeking excursions. Despite their strictly subterranean mode of life, the African mole-rats possess small, superficially located and structurally normal eyes [8,10–12]. Nevertheless, the image-forming vision is compromised due to the properties of the optical apparatus [12], very low visual acuity [8], and, at least in some species, also due to disorganized lens [13] and retinal architecture [14]. Bathyergids have rod-dominated retinae but possess significant cone populations ($\sim$10% of photoreceptors are cones) [10,11]. Nearly all cones express a short-wave-sensitive (S) opsin (commonly blue- or ultraviolet-sensitive in mammals). Many of these S cones co-express small amounts of a middle-to-long-wave-sensitive (L) opsin (commonly green- or yellow-sensitive in mammals), but there are only few pure L cones [11]. Rhodopsin has its absorption peak (λ_{max}

inferred from amino acid sequence) at 496–498 nm [15], the exact spectral tuning of the cone opsins is not known. Paradoxically, the eye seems to be adapted to bright-light rather than low-light conditions. Bathyergids feature small lenses that collect light rather ineffectively (e.g., [8]), high cone proportions [11] and rod nuclei with conventional architecture (Němec et al., unpublished data; cf., [16]). All these features are characteristic of diurnal mammals and are not expected in animals adapted to subterranean darkness.

The central visual system of bathyergids has undergone mosaic regression [17–19]. The only well developed visual domains are those involved in controlling the circadian and circannual biological rhythms – the suprachiasmatic nucleus and the retinohypothalamic projections. The lateral geniculate body (a relay nucleus for cortical perception subserving the detection of colour, form and motion) and pretectum (involved in luminance detection and the pupillary light reflex) are only moderately reduced. By contrast, the superficial visual layers of the superior colliculus (which exerts an important function in object localization) and the accessory optic system (used to stabilize the image on the retina during head movements) are vestigial. This indicates that the bathyergid mole-rats are poorly equipped for the detection and orientation towards objects in the visual field, and for the tracking of moving objects. Thus, their surface activities can hardly be visually-guided.

Taken together, the neuroanatomical findings suggest conservation of basic visual capabilities, casting doubt on the long-lasting notion that the African mole-rats are blind [20,21]. In addition, they show that the visual system of the African mole-rats is neither suited for above-ground spatial orientation nor adapted for low-light vision. Therefore, it has been suggested that the main function of the residual, low acuity vision is to localize breaches in the burrow systems that let in light [7,8,12,22].

In contrast to this wealth of information on organization of bathyergid visual system, almost nothing is known about their visual capacities. Recently, a single study has reported a light/dark discrimination ability in the Zambian mole-rats, *Fukomys anselli/kafuensis* [22]. Likewise, no experimental studies testing the adaptive significance of vision in the context of bathyergid anti-predatory behaviour are available. In this study, we investigated the visual capacities and the role of vision in two other Afrotropical mole-rats, the social giant mole-rat *Fukomys mechowii* and the solitary silvery mole-rat *Heliophobius argenteocinereus*. Firstly, we tested whether these species are able to perceive full-spectrum light and monochromatic lights of different wavelengths. Secondly, we examined their possible colour discrimination abilities. Finally, we tested the hypothesis that vision plays a role in bathyergid anti-predatory behaviour and tunnel maintenance by assessing whether light penetrating into an artificial tunnel system provokes plugging of the illuminated tunnel.

Results

White light avoidance

The mole-rats showed clear heliophobic behaviour. Both species avoided the illuminated box and nested significantly more often in the dark box (*F. mechowii*: $\chi^2 = 7.1$, $P<0.01$, $N = 17$; *H. argenteocinereus*: $\chi^2 = 15.4$, $P<10^{-4}$, $N = 26$; Fig. 1, left bars).

Photoavoidance response to blue and green light

Both species preferred nesting in the dark box and avoided box illuminated by blue (*F. mechowii*: $\chi^2 = 4.8$, $P = 0.029$, $N = 17$; *H. argenteocinereus*: $\chi^2 = 5.0$, $P = 0.025$, $N = 20$) and green-yellow light (*F. mechowii*: $\chi^2 = 5.0$, $P = 0.025$, $N = 20$; *H. argenteocinereus*: $\chi^2 = 5.4$, $P = 0.020$, $N = 15$) (Fig. 1, third and fourth left bars). By contrast, animals showed a random choice between the dark box and the box illuminated by red light (*F. mechowii*: $\chi^2 = 0.06$, $P = 0.81$, $N = 17$; *H. argenteocinereus*: $\chi^2 = 0.2$, $P = 0.65$, $N = 20$; Fig. 1, right bars).

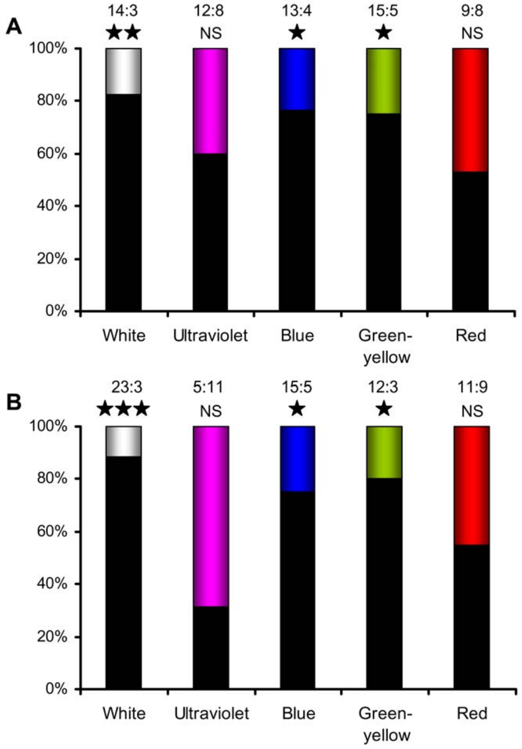

Figure 1. Light avoidance in the giant mole-rat *Fukomys mechowii* (a) and the silvery mole-rat *Heliophobius argenteocinereus* (b). Black bars represent the percentage of choices towards the dark and coloured bars towards the illuminated arm. The absolute number of choices made (dark: illuminated) and the statistical significance level of the response to the corresponding light are shown above each bar (***, $P<0.001$; **, $P<0.01$; *, $P<0.05$; NS, non significant).
doi:10.1371/journal.pone.0011810.g001

No evidence for UV sensitivity

Both species showed a random choice between the dark box and the box illuminated by UVA light (*F. mechowii*: $\chi^2 = 0.8$, $P = 0.37$, $N = 20$; *H. argenteocinereus*: $\chi^2 = 2.25$, $P = 0.13$, $N = 16$; Fig. 1, second left bars). Surprisingly, the animals exhibited a random choice between the blue and UVA illuminated boxes (*F. mechowii*: $\chi^2 = 3.2$, $P = 0.07$, $N = 20$; *H. argenteocinereus*: $\chi^2 = 0.6$, $P = 0.44$, $N = 15$; (Fig. 2, left bars), although trend towards preference of the UVA illuminated box was seen in *F. mechowii*.

No evidence for colour discrimination

In trials where boxes were illuminated by two different monochromatic lights, the mole-rats significantly preferred the box illuminated by red light to the box illuminated by blue light (*F. mechowii*: $\chi^2 = 4.0$, $P = 0.045$, $N = 16$; *H. argenteocinereus*: $\chi^2 = 11.63$, $P<0.001$, $N = 22$; Fig. 2, right bars), but exhibited a random choice between blue and green light (*F. mechowii*: $\chi^2 = 0.28$,

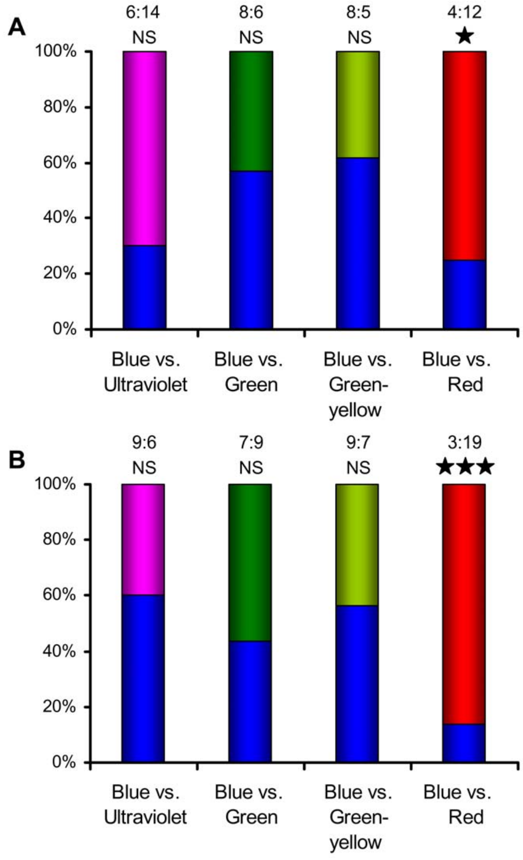

Figure 2. Results of colour preference test in the giant mole-rat *Fukomys mechowii* (a) and the silvery mole-rat *Heliophobius argenteocinereus* (b). Coloured bars represent the percentage of choices towards the respective colours. The absolute number of choices made (blue: other colour) and the statistical significance level of the response to the corresponding choice between two colours are shown above each bar (***, P<0.001; *, P<0.05; NS, non significant).
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$P = 0.59$, $N = 14$; *H. argenteocinereus*: $\chi^2 = 0.25$, $P = 0.62$, $N = 16$) and between blue and green-yellow light (*F. mechowii*: $\chi^2 = 0.69$, $P = 0.41$, $N = 13$; *H. argenteocinereus*: $\chi^2 = 0.25$, $P = 0.62$, $N = 16$) (Fig. 2, middle two bars).

Light induced burrow plugging behaviour

Fukomys mechowii and *Heliophobius argenteocinereus* blocked the illuminated tunnel with peat in 80% and 85% of trials, respectively (Fig. 3). The former species did on average 2.0, the latter species 1.75 plugs per trial. Since the maze consisted of seven blind tunnels, the probabilities that the illuminated tunnel will be blocked by chance are 2.0/7 and 1.75/7, respectively. Consequently, if mole-rats plug blind tunnels accidentally, the blocking of the illuminated tunnel is expected in ~29% and 25% of trials, respectively. Both mole-rat species thus plugged the illuminated tunnel significantly more often than expected by chance (*F. mechowii*: $\chi^2 = 18.4$, $P < 10^{-4}$, $N = 15$; *H. argenteocinereus*: $\chi^2 = 38.4$, $P < 10^{-9}$, $N = 20$). In addition, plugs located within the illuminated tunnel were longer and more tightly packed with peat than those located within the dark tunnels (data not shown).

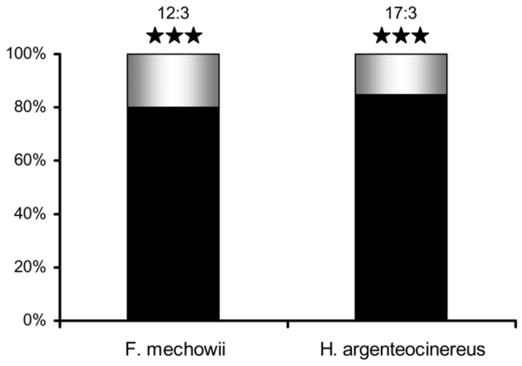

Figure 3. Burrow plugging behaviour. Black and white bars represent the percentage of trials in which experimental animals did and did not plug the illuminated burrow, respectively. The absolute number of trials and the statistical significance level of the response are shown above each bar (***, P<0.001).
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Discussion

The preferential nesting assays performed in this study show that two bathyergid species, the social giant mole-rat *Fukomys mechowii* and the solitary silvery mole-rat *Heliophobius argenteocinereus*, exhibit a clear photoavoidance response to white, blue and green-yellow light, but no significant reaction to ultraviolet or red light. The mole-rats thus retain dark/light discrimination capabilities and a capacity to perceive short to medium-wavelength light. These findings further imply that the mole-rat S opsin has its absorption maximum in the blue rather than the UV part of the spectrum. The assay did not yield conclusive evidence regarding colour discrimination. Finally, the blocking of illuminated tunnels reported here suggests that light serves as a cue signalling the damage of the tunnel system, and therefore points to an important role of vision in bathyergid anti-predatory behaviour and tunnel maintenance.

Dark/light discrimination

Among strictly subterranean mammals, light avoidance behaviour has been reported in five species of African mole-rats [present study, 12, 22], the blind mole-rat *Spalax ehrenbergi* [23], and two species of insectivore talpid moles – *Talpa europea* and *T. occidentalis* – [24–27]. It is notable that *S. ehrenbergi* and *T. occidentalis* posses completely subcutaneous eyes with a degenerated optical apparatus [28–30] and thus represent the extreme cases of eye regression. Consequently, the capacity to distinguish between light and darkness seems to be a common trait amongst subterranean mammals.

Rod and cone opsins, spectral sensitivity and colour discrimination

Bathyergids have a unique photoreceptor mosaic consisting of rods (~90% of photoreceptors), dual pigment cones coexpressing S and L opsins (~7%), pure S cones (~2%) and pure L cones (~1%) [11]. Hence, the mole-rat retina is equipped for both rod scotopic (low light) and cone photopic (daylight) vision. The two spectrally different cone types may subserve dichromatic colour vision, provided that the appropriate post-receptor retinal and cortical circuits for colour processing are also preserved. However, the expression level of the S opsin by far exceeds that of the barely detectable L opsin [11]. The S opsin dominance supports a greater short-wavelength sensitivity than mid-wavelength sensitivity. Nevertheless, the avoidance of both blue and green-yellow light, and a random choice between blue and green light and between blue and green-yellow light demonstrated here, clearly show that that mole-rats are able to see blue as well as green and green-yellow light. These findings raise the question as to whether the green/green-yellow light sensing is L cone- or rod-mediated.

The green light (λmax = 507 nm) irradiance of 5 µmol photons s^{-1} m^{-2} corresponds to a luminance of ~640 scotopic candelas m^{-2}. For a human eye, this luminance is at least 1 log unit higher than that needed for rod saturation [31]. A green light of this intensity produces $\sim 3.72 \times 10^5$ and $\sim 1.16 \times 10^4$ photoisomerizations per rod per second (Rh* rod^{-1} s^{-1}) in the mouse with a fully dilated and fully constricted pupil, respectively [32]. Because the mouse pupil is fully constricted under these light levels [33], the latter estimate is more realistic. The threshold for cone activation is ~30 Rh* rod^{-1} s^{-1} [34]. In the rat, rod saturation occurs at $\sim 4 \times 10^3$ Rh* rod^{-1} s^{-1} [35]. In a transgenic mouse whose retina lacks cones, the ganglion cell response greatly attenuates at the intensity of $\sim 10^4$ Rh* rod^{-1} s^{-1} and disappears at intensities above 10^5 Rh* $rod^{-}1$ s^{-1} [36]. One may speculate that bathyergid mole-rats are even more sensitive to rod saturation caused by bright light than surface-dwelling rodents. Their eyes are smaller than those of mouse, so that an equivalent amount of light passing the murine and mole-rat pupils is spread over a ~3–5-fold smaller area on the mole-rat retina ([37]; Němec et al., unpublished data). Moreover, the rods of mole-rats have substantially larger inner and outer segment diameters when compared to rat or mouse [11]. Consequently, more photons are funnelled to an individual rod in the mole-rats. At the same time, bathyergid rods have shorter outer segments with less densely packed discs (the opsin-containing structures) than sighted rodents [10], suggesting that the total amount of rhodopsin per rod may actually be smaller in bathyergids. As a result, ambient light of any given intensity would bleach a higher fraction of rhodopsin in the bathyergid mole-rats. Assuming that the sizes of fully constricted pupils and photon capture efficiencies of rods are comparable in mouse and bathyergids, one can roughly estimate (using the formulas published by Lyubarsky et al. [32]) that the green light used in this study produces $\sim 1.7 \times 10^5$ Rh* rod^{-1} s^{-1} and $\sim 1.0 \times 10^5$ Rh* rod^{-1} s^{-1} in *H. argenteocinereus* and *F. mechowii*, respectively. Taken together, the light intensity used in our experiments is clearly in the photopic range, where cone signals dominate and rods contribute little, if anything, to the ganglion cell response. It is therefore very likely that L cones and/or dual-pigment cones mediated the perception of green/green-yellow light in our experiments. Thus, the function of both S and L cones seem to be conserved in the African mole-rats.

Animals chose randomly between the dark box and the box illuminated by red light, and preferred red light significantly when had to choose between the boxes illuminated by red and blue light, implying that they cannot see the red light. This result, however, is not surprising. The rodent L cone pigment is commonly green- or yellow-sensitive with λ_{max} somewhere in the range of about 495 to 535 nm (e.g., [38,39]). Indeed, far red light was intentionally used as a control condition for assay validation. The fact that mole-rats did not avoid UVA light is less trivial. The rodent S cone pigment is either UV- or violet/blue-sensitive, depending on the species (e. g., [40,41]). The task, where animals had to make a choice between the dark box and the box illuminated by monochromatic light, brought clear results: the animals avoided blue light but not UVA light. This finding strongly suggests that the bathyergid S cone pigment is violet/blue-sensitive. The other task, where animals had to choose between the boxes illuminated by blue and UVA light, were less conclusive. While an obvious (albeit not significant) bias towards the preference of UVA light was observed in *F. mechowii*, *H. argenteocinereus* exhibited a random choice between blue and UVA light. The interpretation of the latter puzzling result is currently unclear. One possibility is that the S opsin of *H. argenteocinereus* has its λ_{max} in violet and its absorbance spectrum spreads well below 400 nm. But whatever the reason, the data obtained in this study altogether suggest that the bathyergid S opsin is violet/blue-sensitive.

The preferential nesting experiments provided no evidence for colour discrimination. As noted above, both species chose randomly between nest boxes illuminated by blue and green light and between boxes illuminated by blue and green-yellow light. However, this assay is entirely based on the spontaneous motivation to avoid light. While the evidence for heliophobic (or scotophilic) behaviour is compelling ([22], present study), it remains unclear whether mole-rats would spontaneously prefer either monochromatic light if they were capable to discriminate between them. Hence, the negative results of the performed behavioural tests are inconclusive. It has to be noted in this context, that the very fact that the majority of bathyergid cones coexpress S and L opsins may compromise but does not necessarily preclude colour vision. For instance, mice are able to discriminate colours [42] despite having a substantial population of dual pigment cones [43]. A relatively small number of pure S-cones and L-cones may be sufficient to support dichromatic colour vision. Actually, the proportion of 3% for the spectrally distinct cones outnumbers the proportions in many nocturnal species having between 0.5 and 3% cones among their photoreceptors (for overviews, see [40,44]). Conditioning experiments will be needed to assess the capacity for colour vision in the bathyergid mole-rats.

Possible role of vision in the ecology of mole-rats

The African mole-rats have adopted a subterranean mode of life during the early Miocene, if not earlier [9]. Why do the African mole-rats retain basic visual capabilities even after millions of years of underground existence? Despite a remarkable progress in the understanding of the constrains imposed on bathyergid visual capacities by their minute eyes and a reduced visual system (for review, see [7,8]), the biological significance of vision in the natural environment of the bathyergid mole-rats is uncertain. Hypothetically, vision may contribute to the fitness of bathyergids in three ways.

First, it is well known that some subterranean rodents, including the African mole-rats, use light dark cycle as Zeitgeber to which circadian activity is entrained under laboratory conditions [45–51]. However, it is unclear as to whether such light entrainment occurs in the nature. Since light does not penetrate into sealed underground burrows effectively enough to provide a perceivable cue, it is generally expected that strictly subterranean rodents come commonly into contact with light only during forming mounds. However, at least in some species, this activity is rather irregular. For instance, *H. argenteocinereus* cease mound building during dry season [52] and some individuals produce mounds occasionally with no appearance of new mounds for several weeks/months [53]. In this case, the synchronization of circadian activity with the ambient photoperiod would be probably difficult. Therefore some other environmental factors may act as Zeitgeber. Indeed, the results of a recent radiotracking study suggest that soil temperature could be a better predictor of circadian activity rhythms [54]. The fact that c-Fos expression in the suprachiasmatic nucleus is not gated according to the phase of the circadian clock in some social bathyergid species [55,56] also indicates a reduced photic sensitivity of the mole-rat circadian system.

Second, vision might be useful for guidance and/or timing of rare surface activities. Apart from non-recurring events such as natal dispersal, emigration after depleting of food resources, flooding etc., more regular surface activities such as searching for mates during mating season have been recently reported [57]. In *H. argenteocinereus*, paternity analyses demonstrated that burrow systems of mating pairs were several hundred meters away from each other in some cases. The absence of any belowground connection between mate tunnel systems, and a female biased sex ratio imply that aboveground seeking for a mate is a part of the mating strategy at least in one sex [57]. However, as mentioned above (see Introduction), the extremely low visual acuity and severe regression of the visual domains involved in the coordination of visuomotor reflexes render bathyergid above-ground visually guided navigation and predator avoidance ineffective if not impossible.

Nevertheless, vision may be used to optimize the timing of above-ground excursions. Light intensity as well as illuminant spectra change depending on the time of the day. Hence, different photoreceptors are preferentially stimulated at noon, in the twilight or at night. For example, twilight has a higher level of short-wave components than daylight or moonlight [58]. Thus, S cone dominance may possibly optimize the quantal capture and hence cone primary vision at twilight. However, the underground life is associated with markedly different patterns of cone opsin expression and spectral sensitivity among phylogenetically distant taxa [11,59–62]. More detailed information about species-specific frequencies of light exposure and light related behaviour patterns will be required to asses whether S cone dominance confers any selective advantage to the bathyergid mole-rats. Given that cones switch expression from the S to L opsin in some species during early postnatal development (for review, see [63]), it cannot be currently excluded that the S cone dominance is a mere consequence of arrested cone development.

Third and finally, the adaptive significance of vision may be related to an anti-predatory behaviour [7,8,12,22]. The incidence of light may signal that a burrow is damaged by predators (or incidentally by the activity of large herbivores, rains, etc.) and warn the belowground dweller not to approach the opening too closely. Indeed, the very cautious behaviour of *H. argenteocinereus* approaching damaged burrows in the field suggests that mole-rats are aware of burrow violation well before they reach the damaged place (cf. [64]). Many subterranean rodents, including the African mole-rats, react to damage of their tunnels by blocking the broken part with soil. But does light really act as a cue eliciting this behaviour? Beside light, noise from outside, a change in humidity and/or temperature or increased ventilation in the vicinity of the damage may indicate breaches in the burrow. While all these cues may act in synergy in the nature, here we show that the presence of light per se induces very effectively plugging behaviour under laboratory conditions. Accordingly, light was reported to be the primary cue entraining plugging behaviour also in the pocket gophers, phylogenetically unrelated subterranean rodents that posses large eyes [65]. The ease of demonstrating light induced tunnel blocking in a laboratory experiment suggests firm coupling between light stimuli and the plugging behaviour. We therefore conclude that vision does play an important role in bathyergid anti-predatory behaviour and tunnel maintenance.

Materials and Methods

Animals

The silvery mole-rat (*Heliophobius argenteocinereus*, Peters 1846) inhabits southern Kenya, Tanzania, Malawi, southeast D. R. Congo, eastern Zambia, and northern Mozambique; the giant mole-rat (*Fukomys mechowii*, Peters 1881) inhabits northern Zambia, south D. R. Congo, and Angola. Both model species feature very similar ecologies but differ starkly in their life histories: the silvery mole-rat is solitary while the giant mole-rat is a social cooperative breeder. Their biology has been reviewed recently [66,67].

A total of 26 silvery mole-rats and 44 giant mole-rats were used in this study. The silvery mole-rats were wild caught in Malawi in Mpalanganga estate, Zomba (15° 27′S, 35° 15′E), Zomba plateau (15° 20′S, 35° 16′E), and Mulanje - Chipoka (16° 02′S, 35° 30′E) in 2000 and 2005. Some of the giant-mole rats were caught in 1999 in Ndola in Zambia, but the rest was born in captivity. The animals were reared and/or kept in an animal room with moderate temperature (25±1°C) and a 12L/12D light regime at the University of South Bohemia. The silvery mole-rats were housed individually in plexiglass mazes, the families of the giant mole-rats in terrariums. The mole-rats were fed with carrots, potatoes, lettuce, apples, and rodent pellets. Animals at least one year old were tested. Each mole-rat was tested only once in each test condition. The social giant mole-rats were tested in pairs (or threesomes) to avoid stress from isolation. In this species, the availability of experimental animals was periodically influenced by breeding. In the silvery mole-rat, the sample sizes gradually decreased in the course of testing due to the mortality of the experimental animals (this is to be noted in this context that it took three years to perform all experiments).

Ethic statement. All experiments were approved by Institutional Animal Care and Use Committee at University of South Bohemia and Ministry of Education, Youth and Sports (n. 12924/2007-30).

Experiment 1: preferential nesting assay

The abilities to discriminate between full-spectrum ("white") light and darkness, to perceive monochromatic lights of various wavelengths and to discriminate colours were tested using a preferential nesting assay [39,42]. Mole-rats were allowed to choose between a dark box and a box illuminated by full-spectrum light (Fig. 4a), between a dark box and a box illuminated by monochromatic light (Fig. 4b), and between two boxes illuminated by two different monochromatic lights (Fig. 4c), respectively.

A binary-choice apparatus (Fig. 4a-c) was made of plastic and consisted of a cylindrical centre (diameter 22 cm, height 35 cm), an inner cylinder providing opening and closing of the tunnels (diameter 20 cm, height 30 cm), two opposite tunnels (15×8×8 cm) with two terminal boxes (20×20×20 cm). The boxes could be covered with an opaque plastic lid, a translucent Plexiglass lid or an opaque plastic lid with a central opening (5 cm in diameter) for insertion of spectral filters. In every test, the position of lids was swapped after each trial. To begin each experiment, animals were placed into the closed cylindrical centre. Three pieces of carrot, pellets and nesting material (8 strips 25×5 cm of filter paper) were provided. Then the inner cylinder was rotated so that animals could enter and explore the maze. A result was recorded when all nesting material was found in one box (boxes were checked after 60 and 90 minutes). Between trials, the whole apparatus was thoroughly cleaned with ethanol.

Two fluorescent tubes (OSRAM L 58 W/31-830) were used to produce full-spectrum light (400–750 nm) (Fig. 5). The apparatus was illuminated from a distance of 130 cm. The light intensity at

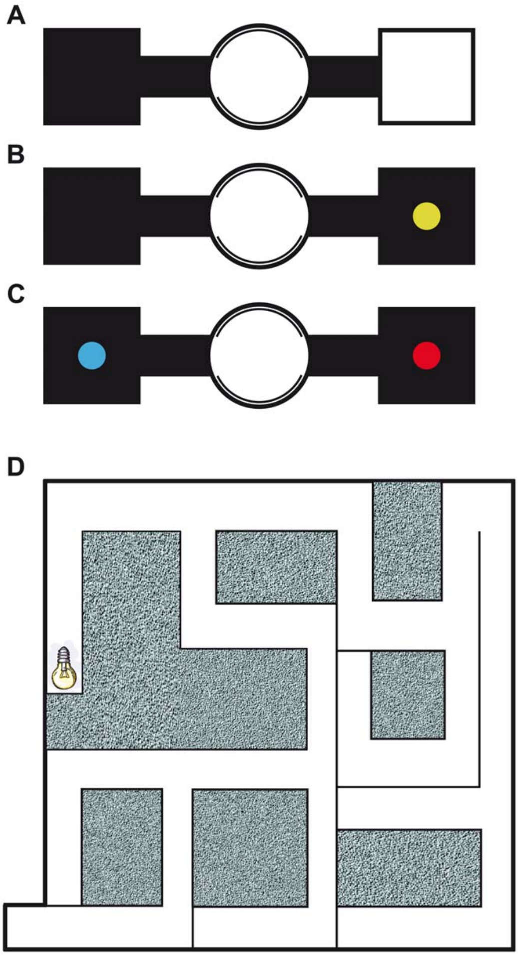

Figure 4. The experimental paradigms used in this study. (a–c) Different layouts of a binary-choice apparatus for testing nest building preference. (d) Diagram of an artificial burrow system used to evaluate burrow plugging behavior; the bulb icon marks the illuminated tunnel. A detailed description of the mazes and experimental protocols are given in the text.
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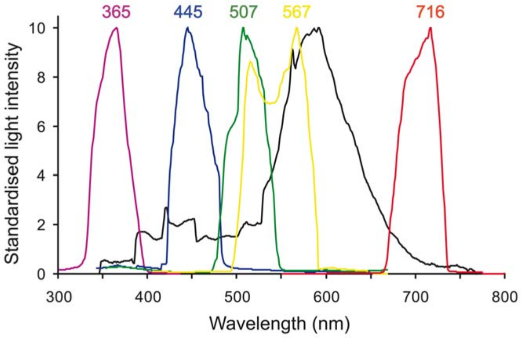

Figure 5. Spectra of the full-spectrum and monochromatic lights used in the experiments. The black line indicates the spectrum of the full-spectrum light produced by fluorescent tubes; coloured lines indicate the spectra of the monochromatic lights (note that the colour-code is symbolic, i.e., the colours do not exactly match to the spectra). The peak wavelengths are given for monochromatic lights. For clarity, all spectra are standardised by taking the maximum value within the measured wavelength interval as 10.
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the bottom of the illuminated sites was 10 µmol photons s^{-1} m^{-2}, as measured by a data logger (Minikon QT, EMS, Czech Republic). To produce monochromatic lights of various wavelengths, 40 W incandescent light bulbs were used in combination with colour filters (diameter 50 mm, thickness 5 mm, Chroma Technology Corp., Rockingham, USA) representing the following parts of the visible spectrum: blue (420–490 nm), green (470–550 nm), green-yellow (495–590 nm) and red (665–735 nm) (Fig. 5). A glass Petri dish filled with cold water was placed above each lid to absorb any heat radiation from the bulbs. The position of the bulb was adjusted according to the type of filter used in order to achieve the light intensity of app. 5 µmol photons s^{-1} m^{-2} at the bottom of each nest box. To produce UVA light (350–400 nm), a UV lamp (UVP, Inc., Upland, USA) was used in combination with a UV-pass filter (Jos. Schneider Optische Werke GmbH, Bad Kreuznach, Germany). The light intensity was set to 1 µmol photons s^{-1} m^{-2} to minimize the possible detrimental effect of UV light on the experimental animals. The intensity of the UV light was measured using a PD300-1W/1Z02411 photodiode sensor (Ophir Optronics Ltd., Israel). Light spectra were determined using Avaspec 2048 Fiber Optic Spectrometer (Avantes BV, Eerbeek, The Netherlands). The temperature in each box was measured after each trial using a probe thermometer (TESTO 425). No difference in temperature was found between boxes. The cylindrical centre of the maze was illuminated by full-spectrum light with an intensity of 10 µmol photons s^{-1} m^{-2} in all experiments.

Experiment 2: burrow plugging behaviour

In this experiment, the reaction of mole-rats to light penetrating into a maze simulating a natural burrow system (Fig. 4d) was tested. If the incidence of light serves as a signal of a damaged burrow and mole-rats are capable of detecting this alert cue visually, they should fill the illuminated part of the maze with substrate.

The maze was made of transparent plexiglass, measured 110×100×10 cm and consisted of seven blind tunnels. The whole maze was tightly closed with a transparent lid. Before the experiment, this lid was covered with black paper except for the end of one blind tunnel that remained transparent. During experiments, this end was illuminated by a 40 W incandescent light bulb from a distance of 130 cm. The animals were introduced into the maze with a thin layer (~1 cm) of horticultural peat and food provided. Subsequently, the maze was covered and the transparent end of the tunnel exposed to light. Each animal (pairs or threesomes in case of the giant mole-rats) spent two hours in the maze. Afterwards, we recorded whether the experimental animals plugged the illuminated tunnel.

Data analysis

In all experiments, we used chi-square tests to analyze the data for a preferential choice. In the Experiment 1, a random choice was asserted by the null hypothesis, i.e., the expected (theoretical) frequency was 1:1. In the Experiment 2, the null hypothesis asserted that the illuminated tunnel in the experimental maze was plugged by chance. The probability the probability of the illuminated tunnel being plugged was calculated as the ratio of the mean number of plugs per trial ($\text{Plug}_{\text{mean}}$) to the total number of the blind tunnels in the maze (there is a one in seven chance that the illuminated tunnel will be plugged). Thus the expected frequency of the illuminated tunnel plugging was computed as follows: $N \times \text{Plug}_{\text{mean}}/7$. Consequently, the expected frequency of the presence: the absence of the plug in the illuminated tunnel was $N \times \text{Plug}_{\text{mean}}/7$: $N \times (1 - \text{Plug}_{\text{mean}}/7)$.

A 95% confidence level ($P<0.05$) was used to judge statistical significance. Analyses were performed using STATISTICA for Windows (StatSoft, Inc., Tulsa, OK, USA).

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Author Contributions

Conceived and designed the experiments: OK RŠ PN. Performed the experiments: OK. Analyzed the data: OK PN. Wrote the paper: OK RŠ PN.

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Chapter 3

Light propagation in burrows of subterranean rodents: tunnel system architecture but not photoreceptor sensitivity limits light sensation range

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Light propagation in burrows of subterranean rodents: tunnel system architecture but not photoreceptor sensitivity limits light sensation range

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Abstract

About 300 species of mammals have adapted to the dark underground ecotope. Despite a long history of underground existence, many strictly subterranean species have retained structurally normal eyes possessing the capability of image-forming vision. Moreover, their retinae often feature high cone proportions, an indication of conserved photopic (daylight) vision. Although it has been suggested that low acuity vision plays an important role in predator avoidance, not a single attempt to measure light conditions in burrows has been made so far. Here, we report the first measurements of light propagation in an illuminated artificial tunnel and in experimentally opened burrows of Ansell's mole-rat, *Fukomys anselli* in its natural habitat. Only about 0.2–2.5% of the ambient visible light entered the opened burrow. Light intensity attenuated quickly and reached mesopic light levels (at which both cones and rods contribute to vision) within a few centimetres from the burrow opening; scotopic light levels (at which only rods operate) were estimated to be reached at one to a few metres from the opening. Thus, although cones may hypothetically contribute to vision for up to a few metres, they play an indispensable role only in the immediate vicinity of a breach, where rods are fully saturated. Rod-mediated light sensation in straight tunnels seems to be possible over distances much longer than 100 m, implying that it is the burrow architecture (tortuosity and branching) what limits light sensation under natural conditions. These findings clearly show that light propagating within a breached burrow may serve as a reliable cue providing information about the site of potential predation risk. Both rods and cones contribute to this signalling. The fact that blue light propagated much less efficiently than longer wavelength light suggests that the short-wave-sensitive opsin dominance in the African mole-rats represents a non-adaptive feature that seems to be associated with arrested cone development.

Introduction

Mammals have adopted various lifestyles associated with different light conditions and visual challenges (Peichl, 2005). About 300 mammalian species have adapted to the stable, low-oxygen and dark underground ecotope (reviewed in Nevo, 1999; Lacey, Patton & Cameron, 2000). Subterranean mammals, which live and forage underground, have small eyes. While some of them such as the marsupial mole (*Notoryctes typhlops*), the blind mole rats (Spalacidae), the golden moles (Chrysochloridae) and some moles (Talpidae) possess minute, subcutaneous eyes with a degenerated optical apparatus and vestigial visual system, the only function of which is to detect ambient light for photoperiod perception, some others, not less adapted to subterranean existence, such as the African mole-rats (Bathyergidae), zokors (Myospalacinae) and some moles (Talpidae) have quantitatively reduced, but structurally normal eyes that have retained the capability of image-forming vision (for review, see Burda, Bruns & Muller, 1990; Němec *et al*., 2007). The role of vision in these strictly subterranean, congenitally microphthalmic mammals is much less obvious. The available anatomical evidence strongly suggests that their visual systems are neither suited for above-ground spatial orientation nor adapted for low-light vision (cf. Němec *et al*., 2007, 2008; Kott, Šumbera & Němec, 2010). Therefore, it has been suggested that besides maintaining circadian rhythms the main function of the residual, low acuity vision is to localize breaches in the burrow systems that let in light and thereby act as an early warning system helping subterranean mammals to avoid predators

(Hetling *et al.*, 2005; Wegner, Begall & Burda, 2006; Němec *et al.*, 2007, 2008). Laboratory experiments have indeed demonstrated light-evoked aversive behaviour in three species of African mole-rats (Hetling *et al.*, 2005; Kott *et al.*, 2010) and in the pocket gophers (Werner, Nolte & Provenza, 2005).

Paradoxically, the eyes of subterranean mammals feature high cone proportions (10–31% of the photoreceptors are cones, reviewed in Peichl, 2005), an indicator of conserved photopic (daylight) vision. Moreover, phylogenetically unrelated subterranean species exhibit markedly different patterns of cone opsin expression (reviewed in Peichl, 2005; Němec *et al.*, 2007), a finding that is not in line with convergent adaptation to subterranean darkness and argues for species-specific adaptation to different visual demands or retention of taxon-specific ancestral photoreceptor properties.

Clearly, more information about the species-specific frequencies of light exposure and about light propagation in burrow systems are needed to assess the adaptive value of a given photoreceptor arrangement and the ecological significance of vision in subterranean mammals. Surprisingly, not a single attempt to measure light conditions in burrows has been made so far. To bridge this gap, we report here the first measurements of light propagation in an illuminated artificial burrow and in experimentally opened burrows of the Ansell's mole-rat in its natural habitat. The artificial burrow was designed to possess poor optical properties. It was made of black plastic and its walls covered with brownish-black horticultural peat, implying that almost all visible light that hit the walls was absorbed. The Ansell's mole-rat burrows, by contrast, featured much better optical properties. The soil was yellowish-brown at the study site. Utilizing light propagation measurements conducted in these optically different burrows, we estimated the maximum distance over which light may be seen in a breached burrow. These estimates were calculated for Ansell's mole-rat using previously published formulas (Lyubarsky, Daniele & Pugh, 2004). This species was chosen as a model because its visual system and role of vision have been studied extensively (e.g. Cernuda-Cernuda *et al.*, 2003; Němec, Burda & Peichl, 2004; Peichl, Němec & Burda, 2004; Wegner *et al.*, 2006; Němec *et al.*, 2008; de Vries *et al.*, 2008). Moreover, similar to other African mole-rats, it exhibits unique short-wave-sensitive opsin (S-opsin) dominance across the entire retina (Peichl *et al.*, 2004). Because the S-opsin seems to be sensitive to blue light in bathyergids (Kott *et al.*, 2010), we compared the propagation of blue light with that of longer wavelengths to assess whether tuning to a predominant sensation of blue light confers any selective advantage for bathyergid mole-rats.

Material and methods

Laboratory measurements

Light propagation/attenuation was measured in an artificial burrow (Fig. 1a). Horticultural peat was stuffed around a pipe (6 cm in diameter) placed in the middle of a tunnel (100 × 9 × 9 cm) made of black plastic. Subsequently, the tunnel was tightly closed with an opaque plastic lid and the pipe was pulled out – in this way, an artificial burrow of peat was created. An area of 5 × 5 cm was left open in the burrow roof and illuminated by a tungsten halogen lamp placed 10 cm above this opening. The light level and spectral composition were measured by a HR4000 High-Resolution Spectrometer coupled to an irradiance probe consisting of the optical fibre and a cosine corrector CC-3-UV-S and analysed with the SpectraSuite Spectrometer Operating Software (all Ocean Optics Inc., Dunedin, FL, USA). The collection area of the optic fibre/probe was 0.12 cm^2, wavelength range 200–1100 nm. Light level measurements and spectral analyses were performed directly below the halogen bulb and at 5, 10, 20, 30, 50, 60 and 65 cm from the burrow opening that let in light. The probe was positioned 5 cm above the burrow floor during all measurements. The readings of absolute radiometric irradiance ($\mu\text{W cm}^{-2}\ \text{nm}^{-1}$) were automatically recalculated into

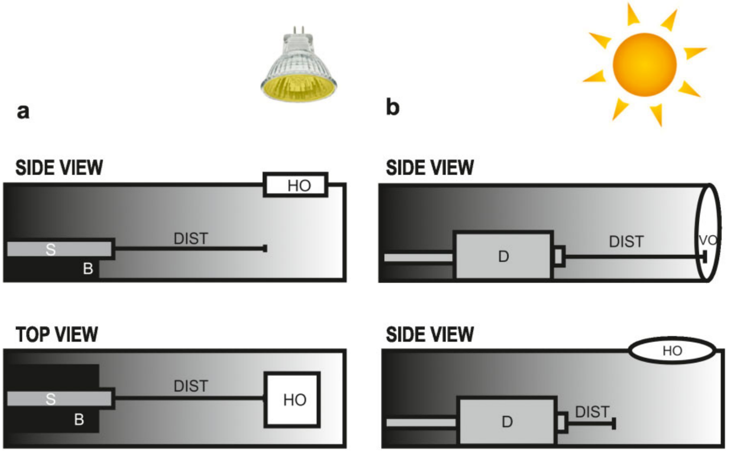

Figure 1 The paradigms used in this study. (a) An artificial burrow used for light level measurements. (b) Measurements of light propagation in Ansell's mole-rat (*Fukomys anselli*) burrows. B, a wooden block serving as a stand for the spectrometer; D, datalogger with embedded quantum sensors; DIST, distance of the datalogger or spectrometer sensor from the illuminated opening; HO, horizontal burrow opening; S, spectrometer; VO, vertical burrow opening.

photon quantities (photon irradiance: photons s^{-1} cm^{-2}) by the above-mentioned software using a bin width of 1 nm. To facilitate quantifications and discussions in terms of quantum catches by different photoreceptor types, the visible spectrum (400–700 nm) was arbitrarily divided into the violet-blue part (400–500 nm), green-yellow part (501–600) and orange-red part (601–700), and in the following text, will be referred to as blue, green and red light, respectively.

Field measurements

Light propagation was measured in natural burrows of the Ansell's mole-rat, *Fukomys anselli* (Bathyergidae, Rodentia) in a miombo woodland in the Lusaka East district, Zambia (15°25′S, 28°17′E) in May 2010. In a randomly chosen burrow system, a straight part of a foraging burrow was separated from the rest of the burrow system by cutting the burrow off to prevent mole-rats from entering it during measurements. Subsequently, vegetation was removed in order to expose the soil to direct sunlight and the burrow was breached: either a horizontally oriented hole was made in its roof or the burrow was transected so that a vertically oriented opening was created at the end of the burrow segment (Fig. 1b). In both cases, the opening was ~5 cm in diameter. Light level was measured inside the burrow at 5, 10, 15, 20, 25, 30, 40 and 50 cm from the burrow opening using a Minikin QT datalogger with in-built quantum sensors and a cosine corrector designed to collect radiation over 180° (Environmental Measuring Systems, Brno, Czech Republic); readings in μmol photons s^{-1} m^{-2}. The collection area of the sensor was 0.25 cm^2, its wavelength range 400–700 nm. Acquired data were processed using the Mini32 software (Environmental Measuring Systems). At each distance from the burrow opening, six measurements were taken at 10-s intervals and averaged. Immediately after the measurements of photon irradiance in the spectral range between 400 and 700 nm, a blue filter passing radiation between 420 and 490 nm (diameter 50 mm, thickness 5 mm, Chroma Technology Corp., Rockingham, NC, USA) was inserted into the opening, plugged all around with soil and the propagation of blue light was measured as described above. These measurements were conducted during sunny days between 10:00 and 12:00 h. In addition, light level was recorded at the distance of 15 cm from the burrow opening every 30 min over the course of 24 h; the measurements were made during full moon nights.

Estimation of light intensities needed for rod and cone activation and rod saturation

The green light ($\lambda_{max} = 507$ nm) luminance of 1 scotopic candela m^{-2} (~ 4.7×10^{11} photons s^{-1} cm^{-2}) produces ~580 and ~20 photoisomerizations per rod per second (Rh* rod^{-1} s^{-1}) in a mouse with a fully dilated and fully constricted pupil, respectively (Lyubarsky *et al*., 2004). The threshold for rod activation is ~ 3.77×10^{-4} Rh* rod^{-1} s^{-1} (i.e. one photoisomerization per 2653 rods; Saszik, Robson & Frishman, 2002). The rod dominated retina of the Ansell's mole-rat contains ~ 400 000 photoreceptors (photoreceptor densities of 100 000–150 000/mm^2, the area of the retina – 3.19 mm^2; Peichl *et al*., 2004; Němec, unpubl. data). Thus, the activation of one out of 2653 rods would result in the activation of ~150 rods per mole-rat retina, a number more than sufficient to sense light (Barlow, 1956). The threshold for cone activation is ~30 Rh* rod^{-1} s^{-1} (Deans *et al*., 2002). In the rat, rod saturation occurs at ~4×10^3 Rh* rod^{-1} s^{-1} (Nakatani, Tamura & Yau, 1991). In a transgenic mouse whose retina lacks cones, the ganglion cell response attenuates greatly at the intensity of ~10^4 Rh* rod^{-1} s^{-1} and disappears at intensities above 10^5 Rh* rod^{-1} s^{-1} (Soucy *et al*., 1998). We have roughly estimated the light intensities needed for rod and cone activation and rod saturation in the Ansell's mole-rat using the following formulas published by Lyubarsky *et al*. (2004):

$$\Phi = Q(\lambda)\tau(\lambda)\frac{S_{\mathrm{pupil}}}{S_{\mathrm{retina}}}a_c(\lambda)$$

where Φ is the average number of photoisomerizations, $Q(\lambda)$ is the time-integrated photon density per unit area in the plane of the pupil, $\tau(\lambda)$ is the transmission of the pre-photoreceptor ocular media for light of the wavelength λ, S_{pupil} and S_{retina} are the areas of the pupil and the retina, respectively and $a_c(\lambda)$ is the 'end-on collecting area' of the photoreceptor.

$$a_c(\lambda) = f\frac{\pi d^2}{4}\left[1 - 10^{-\Delta D(\lambda)L}\right]\gamma$$

where d is the outer segment diameter, $\Delta D(\lambda)$ is the specific axial density of rhodopsin at the wavelength λ, L is the length of the outer segment, γ represents the quantum efficiency of photoisomerization and f is a dimensionless factor that accounts for any light funneling.

As follows from the above equations, the efficiency of conversion of light to photoisomerizations is dependent on the areas of the pupil and the retina, and the average dimensions of the rod outer segment. The following values were used in our calculations: $S_{\mathrm{retina}} = 3.19$ mm^2, $S_{\mathrm{pupil}} = 0.108$ mm^2 and 0.008 mm^2 for a dilated and constricted pupil, respectively, $d = 3.25$ μm, $L = 10$ μm (Peichl *et al*., 2004; Němec, unpubl. data). Because of the extreme responsiveness of the pupil to steady retinal illumination (Pennesi, Lyubarsky & Pugh, 1998), we presupposed that the pupil is fully dilated at the light levels needed for rod and cone activation and fully constricted at the light levels needed for rod saturation. Assuming that optical properties of the pre-photoreceptor media and the photon capture efficiencies of rods are comparable in mouse and bathyergid mole-rats, we have adopted all other factors from Lyubarsky *et al*. (2004): $\tau(\lambda = 500$ nm$) = 0.7$; $f = 1.3$, $\Delta D(\lambda_{max}) = 0.019$ o.d. units μm^{-1}, $\gamma = 0.667$.

Estimation of light sensation range

Because it was not technically feasible to measure light propagation/attenuation over long distances, we determined power functions characterizing the relationship between photon irradiance and the distance from the burrow opening using ordinary least square linear regression. Both mentioned variables were ln-transformed before analysis. Equations

Table 1 Photon irradiance of light propagating within the artificial burrow

	Distance from burrow opening (cm)							
Spectral range (nm)	0	5	10	20	30	50	60	65
400–500	5.10×10^{15}	4.81×10^{12}	2.16×10^{12}	9.79×10^{11}	6.81×10^{11}	1.81×10^{11}	1.26×10^{11}	1.27×10^{11}
501–600	2.04×10^{16}	2.99×10^{13}	1.22×10^{13}	5.36×10^{12}	3.48×10^{12}	8.35×10^{11}	5.59×10^{11}	4.74×10^{11}
601–700	3.13×10^{16}	8.27×10^{13}	3.22×10^{13}	1.30×10^{13}	7.92×10^{12}	1.95×10^{12}	1.30×10^{12}	1.05×10^{12}

Intensities are given in photon quantities (photons s^{-1} cm^{-2}).

derived from the measured data were subsequently used to estimate distances at which irradiance reaches the levels needed for rod activation, cone activation and rod saturation. Spearman's rank correlation coefficient was used to test correlation between photon irradiance and the distance from the opening.

Results

Light propagation in the artificial burrow

The irradiance in the spectral range between 400 and 700 nm reached about 5.67×10^{16} photons s^{-1} cm^{-2} below the halogen lamp. The vast majority of this light was absorbed by the layer of horticultural peat and only about two thousandths of the visible radiation produced by the lamp entered and propagated within the artificial burrow (Table 1, Fig. 2a). Indeed, at the distance of 5 cm from the opening, light intensity was about three orders of magnitude lower than below the light source. Moreover, the spectral composition of the visible radiation in the artificial burrow was shifted towards red light. Blue, green and red light constituted 9%, 36% and 55% of the source visible radiation, respectively. At the distance of 5 cm from the opening, this share was 4%, 25% and 70% for blue, green and red light, respectively. Light intensity attenuated with distance and, at 65 cm from the opening, the irradiance was four to five orders of magnitude lower than below the light source. Blue light propagated less efficiently than long-wavelength light. Green light irradiance remained about 4 to 6 times higher than blue light irradiance throughout the measured segment of the burrow. Red light propagated the farthest and its irradiance remained ten and two times higher than that of blue and green light, respectively.

The irradiance spectra of the light source and of the light propagating within the burrow are given in Supporting Information Fig. S1.

Light propagation in the natural burrow

Peak sunlight irradiance in the spectral range between 400 and 700 nm reached about 7.227×10^{16} photons s^{-1} cm^{-2} on the burrow surface. The amount of sunlight that entered and propagated within the breached burrow depended largely on the opening orientation (Table 2, Fig. 2b). While about 2.5% of the visible solar radiation entered into the transected burrow via the vertical opening, less than 0.3% of the radiation entered the burrow via the horizontally oriented opening. Thus, at the distance of 5 cm from the vertical opening, light intensity was about one order of magnitude higher than at the same distance from the horizontal opening. Light propagated more efficiently in the horizontally opened burrow (Fig. 2b). Blue light (spectral range 420–490 nm) constituted only about 7.5–10% of the visible light that entered the burrow and attenuated quickly. Blue light level reached a light intensity threshold of the used datalogger at 10 cm and 20 cm from the horizontal and the vertical opening, respectively (Table 2, Fig. 2b).

Changes in light level were surprisingly small over the 24 h period (Fig. 3a,b). In the vertically breached burrow, visible light irradiance varied by at most 30-fold, ranging typically between $\sim 2 \times 10^{14}$ and $\sim 2 \times 10^{13}$ photons s^{-1} cm^{-2} during a sunny day and between $\sim 2 \times 10^{13}$ and $\sim 6 \times 10^{12}$ photons s^{-1} cm^{-2} during a moonlit night (Fig. 3a). Blue light irradiance ranged between $\sim 2 \times 10^{13}$ and $\sim 6 \times 10^{12}$ photons s^{-1} cm^{-2} during the day and was $\leq 6 \times 10^{12}$ photons s^{-1} cm^{-2} during the night (Fig. 3a). In the horizontally breached burrow, visible light irradiance ranged between $\sim 7 \times 10^{13}$ and $\sim 10^{13}$ photons s^{-1} cm^{-2} during the day and was $< 10^{13}$ photons s^{-1} cm^{-2} during a moonlit night; the minimum irradiance reached under overcast sky could not be measured due to the limited sensitivity of the datalogger. Likewise, blue light irradiance was below the threshold of the datalogger.

Light sensation range calculated for *F. anselli*

The photon irradiance needed for rod and cone activation in the Ansell's mole-rat is $\sim 6.29 \times 10^5$ and 5×10^{10} photons s^{-1} cm^{-2}, respectively. The photon irradiance needed for rod saturation is $\sim 2.28 \times 10^{14}$ photons s^{-1} cm^{-2}, provided that rod saturation occurs at 10^4 Rh* rod^{-1} s^{-1}. In other words, light levels $> 2.28 \times 10^{14}$ photons s^{-1} cm^{-2} constitute the photopic range, light levels $< 5 \times 10^{10}$ photons s^{-1} cm^{-2} the scotopic range and light levels between 5×10^{10} and 2.28×10^{14} photons s^{-1} cm^{-2} constitute the mesopic range, at which only cones, only rods and both cones and rods contribute to vision, respectively.

Because only two measurements were available for blue light propagating within the natural burrow with the horizontal opening (cf. Table 2), we excluded this case from the analysis. In all other cases, photon irradiance was significantly negatively correlated with the distance from the burrow opening (Spearman's correlation coefficient ~ -1, $P < 0.001$). The power functions relating photon irradiance and the distance from the burrow opening, and distances at which light

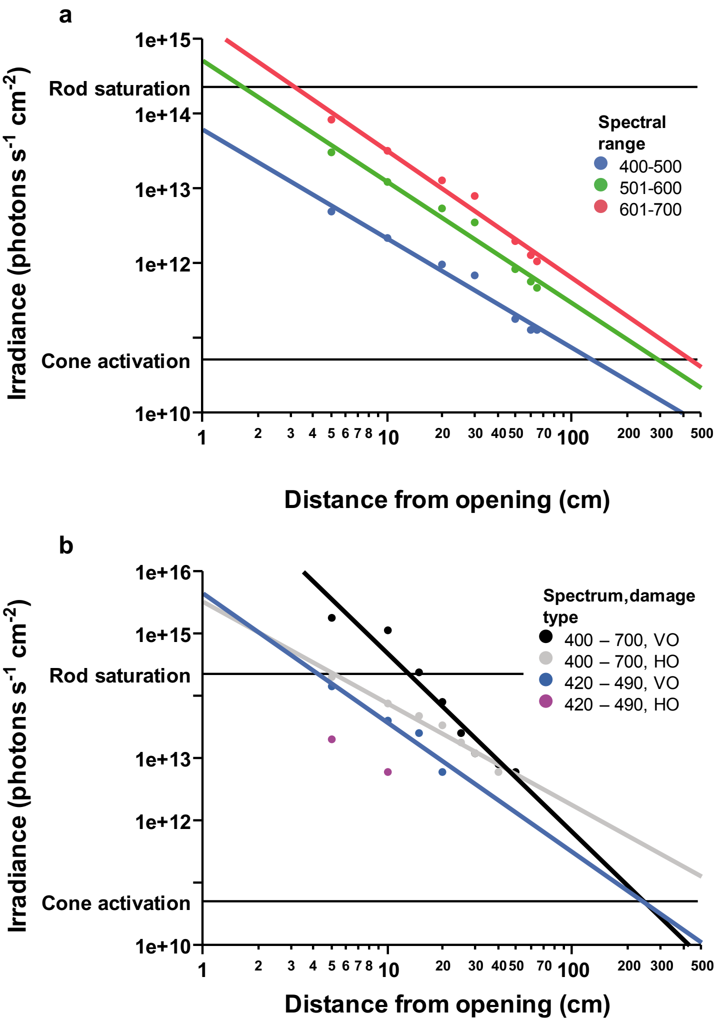

Figure 2 Light propagation within the artificial burrow (a) and the natural burrows of Ansell's mole-rat (b). The two horizontal lines mark light levels needed for cone activation and rod saturation, the zone between them represents mesopic light levels, at which both cones and rods contribute to vision. Above this zone are photopic light levels, at which only cones operate, below scotopic light levels, at which only rods operate. The fitted lines are ordinary least square regression lines. HO, horizontal burrow opening; VO, vertical burrow opening.

Table 2 Photon irradiance of light propagating within the Ansell's mole-rat foraging burrows

Spectral range (nm),	Distance from burrow opening (cm)							
damage type	5	10	15	20	25	30	40	50
400–700, VO	1.82×10^{15}	1.14×10^{15}	2.44×10^{14}	7.83×10^{13}	2.47×10^{13}	1.20×10^{13}	7.83×10^{12}	6.02×10^{12}
400–700, HO	2.01×10^{14}	7.41×10^{13}	4.76×10^{13}	3.37×10^{13}	1.81×10^{13}	1.20×10^{13}	6.02×10^{12}	LOW
420–490, VO	1.39×10^{14}	4.09×10^{13}	2.53×10^{13}	6.02×10^{12}	LOW	LOW	LOW	LOW
420–490, HO	2.05×10^{13}	6.02×10^{12}	LOW	LOW	LOW	LOW	LOW	LOW

Intensities are given in photon quantities (photons s^{-1} cm^{-2}). HO, horizontal burrow opening; LOW, irradiance lower than sensitivity threshold of the datalogger used, VO, vertical burrow opening.

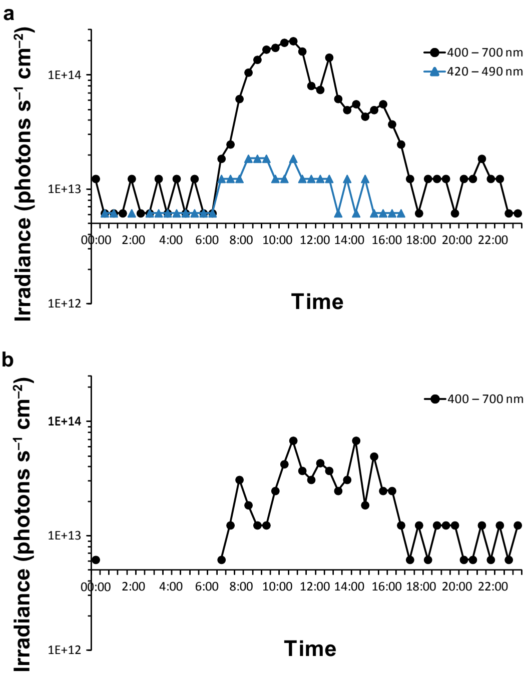

Figure 3 Light level measured over a 24 h period. Measurements were taken 15 cm from the opening in the vertically (a) and horizontally (b) breached burrows. The missing data points represent intensities that were below the threshold of the datalogger used for the field measurements.

intensities reach the above-mentioned critical levels are listed in Table 3.

Because the propagation of moonlight was not measured, light sensation range during a moonlit night cannot be assessed. However, it is notable that, during a clear (not overcast) full moon night, moonlight intensity at 15 cm from the burrow opening was about two and seven orders of magnitude higher than that needed for the activation of cones and rods, respectively.

Discussion

The measurements performed in this study demonstrated a relatively low efficiency of light penetration into breached burrows. Depending on substrate optical properties and damage type, only about 0.2–2.5% of the ambient visible daylight entered the opened burrows. Light intensity attenuated quickly and reached mesopic light levels within a few centimetres from the burrow opening; according to our estimates, scotopic light levels were reached at one to a few metres from the opening. Thus, in the immediate vicinity of a burrow breach, light intensity is in the photopic range, within which only cones operate. Deeper in the burrow, in the mesopic range, both cones and rods contribute to the ganglion cell response. Rod-generated signals become dominant and cone–generated signals diminish with the distance from a burrow breach, although the interactions of the two photoreceptor types are highly complex (cf. Stockman & Sharpe, 2006). Beyond some 1.3–9 m from a burrow breach, in the scotopic range, only rods operate. Taken together, cones may contribute to vision for up to a few metres but play an indispensable role only in the immediate vicinity of a breach, where rods are fully saturated (i.e. the rods output is no longer increasing as luminance increases). Rod-mediated light sensation in straight burrows seems to be possible over distances much longer than 100 m (minimum and maximum estimates are 125 m and 8.9 km, respectively), implying that it is the burrow architecture (tortuosity and branching pattern) what limits light sensation under daylight conditions. Moreover, the 24-h light level measurements reported here show that moonlight propagating within a breached burrow attains levels that are well perceivable. Thus, both daylight and moonlight may serve as a reliable cue providing information about the site of potential predation risk. Finally, the finding that blue light propagated much less efficiently than longer wavelength light suggests that the S-opsin dominance in the African mole-rats represents a non-adaptive feature.

Accuracy of light sensation range estimates

It is to be emphasized that the distance estimates listed above (cf. Table 3) were made for a straight burrow and thus represent a hypothetical maximum distance over which light might be detected. Moreover, being based on irradiances the distances are clearly overestimated. Because the absorbance curves of mole-rat visual pigments are not known, the actual photon catches, representing a better measure of the photoreceptor signal output, could not be calculated. The ranges of photopic and mesopic vision were particularly overestimated in the field measurements as the assessment of their maximum extent is based on the measurement of irradiance in the visible spectral range (400–700 nm), but cones can efficiently absorb only a part of this spectrum (S- and L-cones have their absorption peaks in the blue and green part of the spectrum, respectively). Consequently, the actual quantum catches of both cone types will be significantly lower than the measured total visible irradiance. The same is true for the red light in the artificial burrow. Mole-rat photoreceptor pigments are not tuned to long wavelengths (see above; rhodopsin has its absorption peak in the green part of the spectrum) so the quantum catches of both cones and rods will be quite low for red light even though its absolute irradiance is high. Nevertheless, we argue that these inaccuracies are unlikely to affect the general conclusions of this study. Even if the quantum catch were 10% (which appears to be unrealistically low), the photopic, mesopic and scotopic light levels would be reached at 0 to 6 cm, 27 to 217 cm, and 56 m to 2.17 km, respectively. Since such long straight burrows probably do not exist in nature (Šklíba *et al.*, 2012), light sensation range would remain limited by the burrow system architecture. To further

Table 3 Estimated distances at which irradiance reaches levels needed for rod activation, cone activation and rod saturation

Spectral range (nm), damage type	Power function	Estimated distance of		
		Rod saturation	Cone activation	Rod activation
Artificial burrow				
400–500	$\text{Irr} = 6.270 \times 10^{13} \times \text{Dist}^{-1.436}$	0 cm	132 cm	2961 m
501–600	$\text{Irr} = 5.244 \times 10^{14} \times \text{Dist}^{-1.624}$	2 cm	229 cm	3118 m
601–700	$\text{Irr} = 1.637 \times 10^{15} \times \text{Dist}^{-1.703}$	3 cm*	448 cm*	3374 m*
Foraging burrow				
400–700, VO	$\text{Irr} = 3.591 \times 10^{17} \times \text{Dist}^{-2.869}$	13 cm*	245 cm*	125 m*
400–700, HO	$\text{Irr} = 3.306 \times 10^{15} \times \text{Dist}^{-1.634}$	5 cm*	891 cm*	8886 m*
420–490, VO	$\text{Irr} = 4.496 \times 10^{15} \times \text{Dist}^{-2.077}$	4 cm	243 cm	555 m

Power functions were calculated from the measured light intensities listed in Tables 1 and 2. In all cases, photon irradiance is significantly negatively correlated with the distance from the burrow opening (Spearman's correlation coefficient ~ -1, $P < 0{,}001$). Distances marked by an asterisk are undoubtedly overestimated (for details, see Discussion). Dist, distance from burrow opening (cm), HO, horizontal burrow opening; Irr, photon irradiance (photons s^{-1} cm^{-2}); VO, vertical burrow opening.

support this claim, we have analysed eight fully excavated burrow systems of Ansell's mole-rat (average length of the system 1387 ± 732 m, total length of all systems analysed 11 097 m). While short straight tunnels are quite ubiquitous (70 ± 35 tunnels longer than 1 m per system), the long ones are very rare. The longest straight tunnel was 7.39 m.

Can the results of this study be generalized?

To our knowledge, this study represents the first attempt to quantify the amount of light penetrating into a burrow of any subterranean mammal. However, the distance estimates given above refer to particular conditions under which measurements were taken and should be generalized with caution. The amount of light that enters an opened burrow depends upon many factors such as the surface solar irradiance and incidence angle, burrow architecture and orientation of a burrow opening, optical properties of soil and density of the vegetation cover shading an opening from direct sunlight. Consequently, it is affected by the geographical latitude, elevation, time of day and varies also from locality to locality. The efficiency of light propagation within a burrow also depends on soil properties and the wavelength of the propagating light (see below). Finally, the luminous efficiency of 'subterranean vision' is determined by the visual physiology of a given species. Thus, the only accurate and reliable way of estimating the distance range of vision in an opened burrow is to combine field measurements with the anatomical and physiological examination of the eye of a species in question. However, once again, it is important to note that only the range of the photopic and mesopic zones is dependent on species-specific luminous efficiency of the eye, the maximum light sensation range (i.e. the maximum extent of scotopic zone) is determined by the burrow architecture of a studied species.

Which cone type is best suited for 'subterranean vision'?

The finding that blue light propagates less efficiently than long-wavelength light is not surprising. Since blue light is more scattered in the air than longer wavelengths, the relative probability that blue photons will hit the walls as they propagate within a burrow is higher. Moreover, soil absorbs short wavelengths more efficiently than longer wavelengths. As we know from experience, soil is often black, brown, yellowish or reddish, but seldom blue or green, that is, soil absorbs the vast majority of blue and green light but is prone to reflect yellow, orange and red light. Thus, the wavelength-selective scattering of the air acts in synergy with the wavelength-selective absorption of soil, although the latter is undoubtedly a key factor limiting light propagation in burrows.

Because middle and long wavelengths propagate better than short wavelengths, one would expect L-cone dominance and long-wavelength tuning of L-opsin to be common features in subterranean rodents. However, this is not the case (see Introduction). The blind mole rat, *Spalax ehrenbergi* is the only known species in which the S-opsin is absent and the L-opsin is unusually red-shifted to 534 nm; these photoreceptor properties, however, seem to be associated with the subcutaneous and hence haemoglobin-dominated light environment of the eye (David-Gray *et al.*, 1999). The African mole-rats, by contrast, feature S-opsin dominance across the entire retina (Peichl *et al.*, 2004). Our light propagation measurements demonstrate that this cone arrangement is not suited for vision in burrows. How to explain the persistence of this obviously non-adaptive feature in this strictly subterranean rodent?

S-opsin dominance in bathyergid mole-rats might be a result of arrested cone development. It is known that cones switch expression from the S- to L-opsin in some species during early postnatal development (reviewed in Lukáts *et al.*, 2005). The thyroid hormone receptor $\beta 2$ plays a key role in S-opsin repression and L-opsin activation during cone transdifferentiation (Ng *et al.*, 2001; Yanagi, Takezawa & Kato, 2002). Notably, thyroid Pax-8 knockout mice possess S-opsin-dominated retina (Glaschke, Glösmann & Peichl, 2010). In addition, recent experiments have suggested that thyroid hormone, through its receptor $\beta 2$, not only regulates cone spectral identity during post-natal development but also controls adult cone opsin expression (Glaschke *et al.*, 2011). Methimazole-induced suppression of serum thyroid hormone in adult mice and rats reversibly altered cone patterns by activating the expression of S-opsin and repressing the expression of L-opsin, and the treatment of Pax-8 knockout mice with thyroid hormone restored a wild-type pattern of cone opsin expression (Glaschke *et al.*, 2011). Low levels of thyroid hormones have been reported in the naked mole-rat, *Heterocephalus glaber*, and associated with their subterranean life (Buffenstein *et al.*, 2001). Although it is not known whether low thyroid hormone levels also hold in other bathyergid species, an intriguing possibility that S-cones dominance in bathyergid retina is a mere mechanistic consequence of hypothyroidism certainly deserves investigation.

Role of vision in antipredatory behaviour

As noted in Introduction, it has been suggested that the main function of mole-rat vision is the early localization of breaches in their burrow systems, which in turn plays a key role in burrow maintenance and antipredatory behaviour. Indeed, subterranean mammals usually keep their burrow systems sealed and repair or plug any damaged section quickly. Moreover, it has been shown experimentally that an illumination of a burrow induces its plugging (Kott *et al.*, 2010). Indeed, the available data (Šklíba, Šumbera & Chitaukali, 2008) and experience with mole-rat trapping suggest that mole-rats are very cautious around damaged parts of burrow systems, suggesting that light penetrating into damaged burrows serves as a cue triggering antipredatory behaviour.

The present study clearly shows that the amount of light that penetrates into a damaged burrow system during the day and also during a moonlit night suffices to inform an underground dweller about the site of danger. The sensitivity of rod-mediated scotopic vision is so high that it is only the burrow tortuosity and branching what limits light sensation range under daylight conditions.

We cannot exclude that some other cues (e.g. air current, noise from the outside and change of temperature around a burrow breach) are also relevant to inform a burrow inhabitant about the exact position of burrow damage (cf. Werner *et al.*, 2005). However, we do not think that these factors are of the same importance as light. Sensing ventilation is limited to the immediate vicinity of a breach, provided that the tunnel is not opened at two/multiple places some distance apart. A change of burrow temperature also seems to be less informative because it has certain latency and is only apparent over a very short distance. Nevertheless, the actual role of these environmental stimuli remains to be assessed in standardized laboratory experiments.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. The irradiance spectra of the light source and of the light propagating within the artificial burrow.

Chapter 4

Social and Environmental Influences on Daily Activity Pattern in Free-Living Subterranean Rodents: The Case of a Eusocial Bathyergid

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Social and Environmental Influences on Daily Activity Pattern in Free-Living Subterranean Rodents: The Case of a Eusocial Bathyergid

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Abstract Predictable daily activity patterns have been detected repeatedly even in mammals living in stable environments, as is the case for subterranean rodents. Whereas studies on activity of these rodents under laboratory conditions almost exclusively have concerned themselves with the influence of light, many field studies have revealed signs of an association between the activity pattern and daily fluctuations of temperature under the ground. This would assume that behavioral thermoregulation is probably involved. The only exceptions to the relationship between temperature and activity are 2 eusocial mole-rats of the genus *Fukomys* (Bathyergidae, Rodentia), which indicates that activity patterns could be affected also by social cues. To better understand how social and environmental factors influence the activity pattern in a eusocial mole-rat, we monitored the outside-nest activity in another species of this genus, the Ansell's mole-rat (*Fukomys anselli*), which has a relatively small body mass, high conductance, and more superficially situated burrows. Its daily activity had 1 prominent peak (around 1400 h), and it was tightly correlated with the temperature measured at depth of foraging burrows. Since *F. anselli* has high thermoregulatory requirements to maintain stable body temperature below the lower critical temperature, we conclude that the observed pattern is probably the result of minimizing the cost of thermoregulation. There were no significant differences in the daily activity patterns of breeding males and females and nonbreeders. Members of the same family group tended to have more similar activity patterns, but consistent activity synchronization between individuals was not proven. From the comparison of available data on all subterranean rodents, we assume that social cues in communally nesting mole-rats may disrupt (mask) temperature-related daily activity rhythms but probably only if the additional cost of thermoregulation is not too high, as it likely is in the Ansell's mole-rat.

Keywords *Fukomys anselli*, subterranean rodent, daily activity pattern, radio-telemetry, Bathyergidae, eusociality, circadian rhythms

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Most mammals display circadian rhythms of activity and rest, which ensure that activity takes place during an optimal period of the day in terms of food availability, predation risk, and climatic conditions (Bartness and Albers, 2000). In mammals living in a stable environment with lack of external cues that would potentially reset their circadian clocks, we would expect circadian activity rhythms to be weak or absent. Examples of such mammals are subterranean rodents, that is, rodents that forage underground and rarely emerge from their sealed burrows. The subterranean ecotope is dark and thermally buffered, and neither food availability nor predation risk is thought to substantially vary there during the course of a 24-h cycle (Nevo 1999). It is therefore surprising that many field studies regarding subterranean rodents have exhibited predictable daily activity patterns (e.g., Jarvis, 1973; Gettinger, 1984; Benedix, 1994; Šklíba et al., 2007; Lövy et al., 2013). Some authors have suggested that there is a relationship between activity patterns and daily temperature cycles in the depth of the burrows, even though its amplitude is often very small (typically <4 °C). There are few laboratory data dealing with the effect of temperature on the activity pattern in these animals (cf. Goldman et al., 1997), which contrasts with many studies testing merely the influence of the photoperiod (e.g., Oosthuizen et al., 2003, and citations therein).

The relationship between activity patterns and daily temperature fluctuations that has been detected in some free-living subterranean rodents has the character of either preference or avoidance of high or low temperatures, depending on the species, latitude, or the season, as can be illustrated by the following examples. Whereas blind mole rat *Spalax ehrenbergi*, radio-tracked during the hot Mediterranean summer, minimized its activity during the hottest period of day (Rado et al., 1993), the silvery mole-rat *Heliophobius argenteocinereus*, radio-tracked in Afrotropical woodland in the coldest period of year, showed a positive correlation between temperature and activity (Šklíba et al., 2007). Another subterranean rodent, the plains pocket gopher *Geomys bursarius*, was even found to reduce its activity in both the hottest and the coldest period of the day, resulting in a bimodal activity pattern (Benedix, 1994). The author of the latter study proposed that by displaying such an activity pattern, the animals 1) reduce additional energetic expenses of thermoregulation when the burrow temperature is low and 2) reduce the threat of hyperthermia when it is high. This statement actually represents 2 hypotheses. So far, neither of them has been addressed in any subterranean rodent under laboratory conditions, and it might be technically difficult to do so. Nevertheless, examining temperature-related daily activity patterns across different subterranean rodents in the field and coupling them to available physiological data can help us to evaluate plausibility of such hypotheses.

African mole-rats (Bathyergidae) are a family of strictly subterranean rodents of which the majority of the species are highly social, living in multigenerational family groups. This provides us with an opportunity to study the effect of sociality in addition to environmental factors on these animals' daily activity patterns. Strong social influence on behavioral rhythms is common in social animals living in constraining environments, but it can be expressed in different ways, such as synchronization, desynchronization, or individually variable response to social cues depending, for example, on dominance status (Favreau et al., 2009). In highly cooperative mole-rat family groups, we would expect desynchronized rather than synchronized activity patterns, presumably enabling more effective cooperation in search for food and territory maintenance. This is also in accord with most laboratory observations (Bennett, 1992; Riccio and Goldman, 2000; Schielke et al., 2012; but see Davis-Walton and Sherman, 1994, for signs of activity synchronization in naked mole-rats). Field-based activity data are available from only 2 of the social species, Damara mole-rat *Fukomys damarensis* and giant mole-rat *Fukomys mechowii*, each represented by a single study dealing with a single family group (Lovegrove, 1988; Lövy et al., 2013). Both species engaged in many short activity bouts per day and showed no clear signs of activity synchronization between individuals, but none of them displayed a temperature-correlated daily activity pattern. The authors of the latter study concluded that the similarity of the activity patterns of *F. mechowii* and *F. damarensis* might be, to some extent, a consequence of similar social behaviors in these 2 species, such as use of communal nests and the need for cooperation while performing tasks, overlying the effect of environmental factors.

The 2 above-mentioned species, *F. mechowii* and *F. damarensis*, represent the largest social mole-rats (Bennett and Faulkes, 2000), inhabiting large burrow systems with deep (and therefore more temperature buffered, see Bennett et al., 1988) nests and primary burrows (Lovegrove and Painting, 1987; Šumbera et al., 2012). These factors can potentially contribute to their activity pattern not being markedly affected by the daily temperature cycle. Nevertheless, in smaller-sized social bathyergids, especially in those whose burrow systems lack deep primary burrows, some behavioral response to daily temperature might be energetically advantageous. This likely would be expressed as a predictable daily activity pattern related to the temperature cycle in the depth of the foraging tunnels, which would be similar in most individuals across different family groups.

To better understand the relative importance of social and environmental cues for the mole-rat activity pattern, we chose to study the activity of the Ansell's mole-rat *Fukomys anselli* (Burda et al., 1999), an eusocial species (cf. Patzenhauerová et al., 2013) from Zambia, under natural conditions. This species has a relatively small body mass, and its burrow systems generally lack deeper primary burrows (see Šklíba et al., 2012). We recorded a pattern of outside-nest activity in 17 radio-collared individuals of 5 family groups in the cold dry season. Aims of the study were 1) to reveal whether there is a daily rhythm of activity in this species under natural conditions of the cold dry season; 2) to find whether activity is related to the daily temperature cycle in the depth of foraging burrows, as detected in some solitary subterranean rodents, or whether the relationship is absent, as found in larger-bodied social mole-rats; 3) to reveal how the activity pattern is affected by group affiliation, sex × reproductive status, and body mass and what is the relative influence of these factors; 4) to test whether the activities of individuals from the same family group tend to be rather synchronized or desynchronized; and 5) to review the incidence of likely temperature-related daily activity patterns in subterranean rodent species within the context of relevant physiological parameters of the respective species.

MATERIALS AND METHODS

Studied Animals

The Ansell's mole-rat is a small-sized social species inhabiting a mesic area close to the Zambian capital Lusaka. In the study population, the body masses of adults (≥50 g) were 72 ± 14 g (maximum 96 g) for males and 62 ± 8 g (maximum 83 g) for females. Family groups of up to 13 individuals inhabited extremely large and complicated burrow systems, with the majority of the tunnels located at a depth (from the bottom of the burrow to the soil surface) of around 11 cm (foraging tunnels). A single communal nest per family group was located 47 ± 17 cm (range, 25-90 cm) underground (Šklíba et al., 2012).

Study Locality

The study was conducted in the Lusaka East Forest Reserve in Zambia (15°28′S, 28°25′E, altitude 1320 m), which is covered by natural open-canopy miombo woodland. The climate in Zambia is characterized by 3 seasons: a rainy season (October/November to March/April), a dry cold season (April to July), and a dry hot season (August to October). Average annual rainfall in Lusaka is 809 mm, and on average 5.4 months per year have more than 25 mm of precipitation (http://www.ncdc.noaa.gov/ghcnm/, accessed May 2012).

Fieldwork

Mole-rats were captured by use of Hickman traps. Captured individuals were weighed, their sex was determined, and they were examined for their reproductive status. We considered breeding males as those with conspicuous head muscularity, pigmented corners of the mouth, and conspicuously large testes; breeding females were recognized by perforated vagina and enlarged teats (the breeding status was later confirmed by use of genetic methods, see Patzenhauerová et al., 2013). All animals weighing more than 45 g were anesthetized for a short time by ketamine and xylazine, fitted with radio-collars (brass collar, Pip transmitter; Biotrack Ltd, Dorset, UK), and released back into the burrows where they were trapped within 48 h from capture. The weight of each radio-collar was 2.3 g (<5% of the body mass of the smallest animals under study). Radio-tracking started 3 days after the release of the last animal.

The collared animals were radio-tracked in 2 continuous 96-h sessions. The first session (9-13 May 2010) involved 17 mole-rats from 5 family groups (named P01, P02, etc.; Table 1), which was reduced to 13 individuals in the second session (25-29 May 2010) due to the loss of some collars. Both sessions were in the cold dry season. We used the IC-R20 receiver (Icom America Inc., Kirkland, WA) and a 3-element handheld Yagi antenna to locate the animals. Position of the animals was fixed hourly starting at 0600 h. Since the animals of the same group used the same nest, these places were checked first, and then the animals that were not present here were carefully approached to a distance of 1 to 4 m and fixed. After the end of radio-tracking, all members of the examined family groups were recaptured and the complete burrow systems were excavated and mapped as a part of another study (see Šklíba et al., 2012, for complete information about group sizes, burrow system parameters, ecological parameters, etc.). Handling live animals was in accord with the Declaration of Helsinki and the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Temperatures of the soil surface and at depths of 10, 20, 30, 60 and 120 cm were recorded every 10 min over the whole period of radio-tracking using temperature loggers (Comet System s.r.o., Rožnov pod Radhoštěm, Czech Republic). The depth of 10 cm was chosen to approximate the depth of mole-rat foraging tunnels (Šklíba et al., 2012); the other depths were selected to illustrate lag and attenuation of the temperature fluctuation as a function of the distance from the soil surface. We selected a relatively exposed site for measuring the temperatures, because most of the area of the mole-rat territories was also relatively exposed to the sun. For comparison we placed 1 additional temperature logger at a depth of 10 cm at a site shaded by dense shrubs and trees.

Data Processing

To determine daily patterns of activity of the radio-tracked mole-rats, each radio-fix was designated as either inside or outside the nest. From our previous telemetry studies of mole-rats (Šklíba et al, 2007, 2009), we estimate the accuracy of our fixes at ±0.5 m; thus, all fixes within a 0.5-m radius of the nest were treated as inside the nest. For each animal, an actogram of the outside nest activity was created based on the primary dataset containing the hourly presence or absence of an individual inside the nest. This dataset was also used to test the relationship of outside-nest activity with environmental factors and to test the activity synchronization of individuals within the same group. Subsequently, radio-fixes of each individual from each 96-h radio-tracking session were grouped into twelve 2-h blocks, and the proportion of fixes outside the nest was determined for each of the blocks. This dataset was used to compare activity patterns between individual mole-rats using a multivariate method.

Statistical Analyses

Data throughout the text are provided as mean ± SD. Generalized linear mixed models (GLMMs) were used to test the effect of various environmental factors to activity—that is, presence outside the nest. Presence outside the nest was the dependent variable with binomial distribution. The factors tested (explanatory variables) were light-dark 24-h cycle (categorical predictor: the light hours [0600-1800 h] marked as 1, dark hours [1800-0600 h] marked as 0) and temperatures at various depths (continual predictors; surface, 10, 20, 30, and 60 cm). The temperature at 120 cm underground was not included since its daily amplitude was close to the resolution of the temperature loggers. The 17 radio-tracked individuals were grouped into the following 9 subsets reflecting their sex, reproductive status, and group affiliation: (1) breeding males, (2) breeding females, (3) nonbreeding males, (4) nonbreeding females, and (5-9) members of the respective family groups. Due to the unequal sample size in the categories and their overlap, GLMMs were performed separately for each of the categories. In all GLMMs, both the individuality of a given animal and the radio-tracking day (1-8) were treated as random factors to avoid pseudoreplications. We used Bonferroni procedure to correct the significance level of the tests since we tested the 6 factors separately ($\alpha = 0.05 / 6$). All the tests were performed using R statistical software (R Development Core Team, 2013) extended with the lmer4 package (Bates et al., 2013), which is designed for models with incorporated random factors.

Partial effect of 3 variables (body mass, sex × reproductive status, and group affiliation) on the mole-rat activity patterns (defined by proportions of outside-nest fixes in the twelve 2-h blocks of the 24-h day) was computed by variance partitioning technique (Lepš and Šmilauer, 2003) using the redundancy analysis (RDA). Sex × reproductive status and group affiliation were expressed as sets of 4 and 5 binary ("dummy") variables, respectively. The RDA was performed with the software package CANOCO for Windows, version 4.52 (Microcomputer Power, Ithaca, NY) (ter Braak and Šmilauer, 2002), with the proportions of outside-nest fixes arcsine transformed and all variables entering the analyses centered (to have zero mean). To avoid pseudoreplications (treating 2 sessions of the same individual as independent), the variance partitioning was performed with data from the first radio-tracking session only.

To test whether the outside-nest activity of individuals of the same group is synchronized, we developed a simple permutation test. For each group and radio-tracking session we computed score x representing total number of cases of any dyad of individuals located outside the nest at the same time. Then we randomly exchanged the order of the 4 days of the 96-h session in each individual while maintaining the daytime of the radio-fixes unchanged. Score x was then compared with the score computed for the permutated data (x_p). The proportion of cases out of 1000 permutations where $x_p \geq x$ was set as the significance level of the null hypothesis that the outside-nest activity of the group members is not synchronized within their overall activity pattern.

RESULTS

Characteristics of the radio-tracked mole-rats are presented in Table 1; their actograms are depicted in Figure 1. All individuals except 1 breeding male (M967) performed a single daily peak of activity, usually between 1100 and 1600 h. There was no apparent temporal shift of the activity peak between the 2 radio-telemetry sessions. In both of them, the activity was culminating (i.e., the most animals were active) around 1400 h (Figs. 1 and 2a). Besides demonstrating a peak at about 1400 h, the breeding male M967

Table 1. Characteristics of radio-tracked mole-rats.

Group ID	Animal ID	Sex and Reproductive Status	Body Mass, g	No. of Radio-Tracking Sessions
P01	M038	Breeding M	86	1
	M150	M	84	1
	M203	M	57	2
	F217	Breeding F	69	1
P02	F138	F	56	2
	M967	Breeding M	90	2
P04	F027	F	64	2
	F465	F	68	2
	F485	F	83	2
P05	F419	F	57	2
	F443	F	49	2
	F532	Breeding F	54	2
P10	F015	F	62	2
	M062	Breeding M	87	2
	M092	M	58	2
	M352	M	47	2
	F568	F	56	1

F = female; M = male.

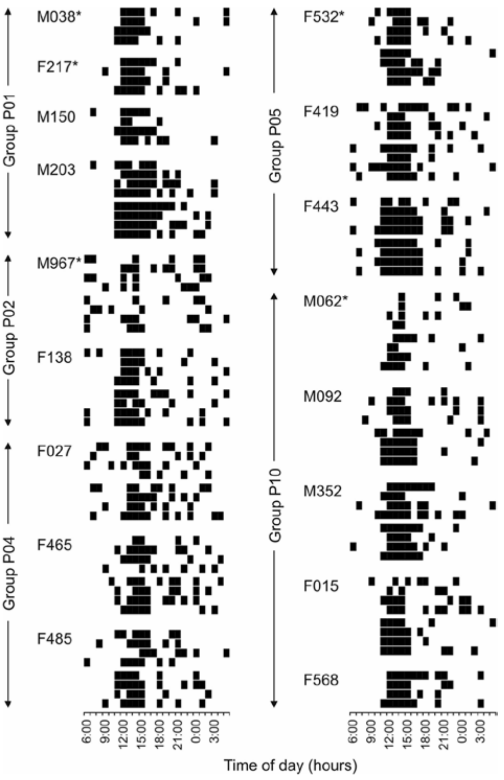

Figure 1. Actograms of 17 radio-tracked mole-rats. Dark bars represent activity (occurrence outside the nest). Every block of 4 lines represents 1 continuous 96-h radio-tracking session of a particular individual. Asterisks mark breeding individuals.

performed 2 additional daily activity peaks—in both radio-tracking sessions around 0100 h and 0600 h.

Mean daily temperatures at different depths are depicted in Figure 2b. At a depth of 10 cm, which corresponds with the depth of the Ansell's mole-rat foraging tunnels, mean daily temperature was 20.9 °C; mean daily minimum and maximum temperatures were 17.5 and 25.5 °C, respectively; and mean daily temperature span was 8.0 °C. In the shaded location, the mean daily temperature at a depth of 10 cm dropped to 18.2 °C and the daily temperature span decreased to 1.8 °C. The outside-nest activity in Ansell's mole-rats was best explained by the temperature cycle at a depth of 10 cm. Of all 6 explanatory variables, this variable explained the largest proportion of variability in the activity in each of the 9 subsets of individuals defined by sex, reproductive status, and group affiliation (Table 2). The effect of the light-dark cycle, although also significant, had markedly less explanatory power. In reproductive males, a markedly lower proportion of explained variability in the activity data indicates a less pronounced effect of all variables examined.

The amount of variability in the activity patterns explained by sex × reproductive status, body mass, and group affiliation was significantly higher than zero only for the dataset where both radio-tracking sessions were combined—that is, including pseudoreplications (Table 3). Group affiliation explained the largest part of the variability of the data, which again was significant only for the dataset containing both radio-tracking sessions.

Outside-nest activity of group members was significantly synchronized only in the first session of group P01 and second session of group P04 (permutation test, $p = 0.003$ and 0.006, respectively).

DISCUSSION

The Ansell's mole-rat was previously reported to have "a weak circadian clock" based on a study of singly-housed captive individuals under artificial light regime (De Vries et al., 2008) and only a "tendency towards rhythmicity" in locomotory activity

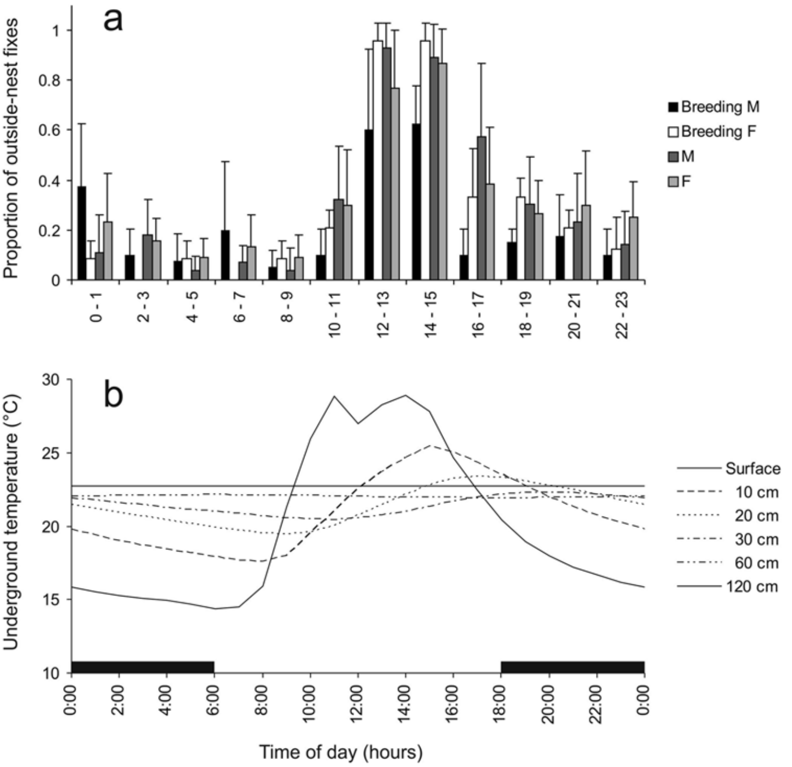

Figure 2. Outside-nest activity of the radio-tracked mole-rats (a) and underground temperature fluctuations (b) during a 24-h day. Means ± standard deviations are presented for the proportions of outside-nest fixes. Black horizontal bars indicate the dark phase of the day. F = female; M = male.

based on monitoring of captive family groups under natural (European) light cycle (Schielke et al., 2012). On the contrary, the activity patterns recorded under natural conditions in the present study showed a clear 24-h periodicity in most individuals (Fig. 1). Since the pattern was similar in all 5 family groups studied and all individuals irrespective of body mass, sex, and reproductive status, we assume that it was determined by an external cue. As expected, of the external factors tested, the best predictor of outside-nest activity appeared to be temperature fluctuations at depth of the foraging tunnels.

An animal activity pattern under natural conditions can differ considerably from the one observed in a laboratory, as illustrated by an example of the coruro *Spalacopus cyanus*, which is nocturnal in the laboratory (Begall et al., 2002) but diurnal in the field (Urrejola et al., 2005). Nevertheless, the dissimilarity between activity patterns of captive (De Vries et al., 2008; Schielke et al., 2012) and free-living (present study) *F. anselli* is striking for 2 reasons. First, the cycle of light, which was also involved in the abovementioned laboratory studies, is generally thought to be a stronger and more universal zeitgeber than temperature fluctuation in vertebrates (Refinetti, 2006). Even in the strictly subterranean *S. ehrenbergi*, a larger proportion of captive individuals entrained circadian activity rhythms by the light cycle rather than by the temperature cycle (Goldman et al., 1997). Second, in a highly social species, the activity patterns can be desynchronized or disturbed by social cues (Riccio and Goldman, 2000; Lövy et al., 2013). Displaying a distinct and relatively uniform activity pattern by free-living Ansell's mole-rats across different family groups (not performed by captive ones housed either singly or in groups) is therefore probably advantageous in terms of survival or energetic balance.

Activity of subterranean rodents includes active digging, which, especially in hard soils, requires a manifold increase in the metabolic rate. This suggests a high potential for heat production and presumably a risk of hyperthermia (Vleck, 1979). We would therefore expect that subterranean rodents would prefer to be active during parts of day when the ambient temperature is lower than average. Exactly the opposite was found in our study of the Ansell's mole-rat. What could be the reason for this behavior?

It is clear that the energetically costly activities, such as excavation of burrows, pushing soil within the burrows, and pushing soil aboveground, are probably not performed throughout the total time an individual spends outside the nest. Exploration or

Table 2. Relationship of outside-nest activity of the radio-tracked Ansell's mole-rats to environmental variables as revealed by the generalized linear mixed model with binomial distribution.

	Temperature, °C					
	Surface	10 cm	20 cm	30 cm	60 cm	Light-Dark Cycle
Breeding M ($n = 3$)	5.4 (25.9) $p < 0.0001$*	6.3 (30.7) $p < 0.0001$*	1.4 (5.9) $p = 0.016$	0.7 (1.1) $p = 0.161$	0.4 (0.6) $p = 0.455$	2.2 (9.1) $p = 0.002$*
Breeding F ($n = 2$)	26.5 (90.8) $p < 0.0001$*	31.8 (108.7) $p < 0.0001$*	8.7 (29.7) $p < 0.0001$*	0.5 (1.8) $p = 0.205$	0.7 (2.9) $p = 0.123$	8.7 (29.9) $p < 0.0001$*
M ($n = 4$)	21.6 (179.2) $p < 0.0001$*	29.0 (241.2) $p < 0.0001$*	8.8 (71.7) $p < 0.0001$*	0.7 (3.4) $p = 0.056$	8.2 (66.9) $p < 0.0001$*	9.1 (74.3) $p < 0.0001$*
F ($n = 8$)	11.7 (211.2) $p < 0.0001$*	15.5 (279.4) $p < 0.0001$*	5.0 (89.8) $p < 0.0001$*	0.1 (1.3) $p = 0.176$	5.2 (93.3) $p < 0.0001$*	4.0 (71.8) $p < 0.0001$*
P01 ($n = 4$)	22.4 (132.0) $p < 0.0001$*	32.7 (193.9) $p < 0.0001$*	11.0 (63.1) $p < 0.0001$*	0.8 (1.6) $p = 0.193$	3.5 (17.9) $p < 0.0001$*	8.2 (74.3) $p < 0.0001$*
P02 ($n = 2$)	3.5 (16.0) $p < 0.0001$*	3.6 (16.6) $p < 0.0001$*	0.4 (1.4) $p = 0.199$	0.6 (2.5) $p = 0.109$	0.1 (0.24) $p = 0.624$	2.1 (9.3) $p = 0.002$*
P04 ($n = 3$)	6.2 (44.2) $p < 0.0001$*	12.3 (88.3) $p < 0.0001$*	6.4 (46.1) $p < 0.0001$*	0.2 (1.2) $p = 0.278$	1.0 (7.1) $p = 0.008$	1.4 (9.6) $p = 0.002$*
P05 ($n = 3$)	21.4 (155.4) $p < 0.0001$*	22.0 (160.0) $p < 0.0001$*	4.8 (34.2) $p < 0.0001$*	1.1 (6.8) $p = 0.009$	0.8 (5.0) $p = 0.029$	10.2 (73.6) $p < 0.0001$*
P10 ($n = 5$)	18.0 (174.2) $p < 0.0001$*	20.9 (204.0) $p < 0.0001$*	5.8 (53.3) $p < 0.0001$*	0.9 (4.4) $p = 0.038$	6.4 (59.3) $p < 0.0001$*	6.1 (55.9) $p < 0.0001$*

The animals are grouped according to their sex and reproductive status and family group affiliation. For each variable, percentage of explained variability and χ^2 (in parentheses) is presented. Statistically significant results after Bonferroni correction ($p < 0.008$) are marked with asterisks; df and χ^2 df in all models are 4 and 1, respectively). F = female; M = male.

Table 3. Quantification of the effects of the 3 variables on the temporal activity pattern of the Ansell's mole-rat as computed by the variance partitioning technique (Lepš and Šmilauer, 2003) using the redundancy analysis with the data set containing only the first radio-tracking session.

	Explained Variance, %	% of Explained Variance	*F*	*p*
All variables	56 (40)	100 (100)	1.3 (1.8)	0.2 (0.006)
Partial effect of group affiliation	30 (19)	54 (47)	1.4 (1.7)	0.2 (0.04)
Partial effect of sex × reproductive status	12 (7)	21 (17)	0.7 (0.8)	0.8 (0.7)
Partial effect of body mass	3 (3)	5 (8)	0.5 (1.1)	0.8 (0.4)
Shared effect	11 (11)	20 (28)		

Values in parentheses are computed with the data set containing both radio-tracking sessions.

patrolling the burrow systems, as well as activities such as feeding and carrying small food items into food stores, could also take considerable time. Nevertheless, most digging and soil pushing activities still occurred during a period of high daily temperature at depths of the surface tunnels (J. Šklíba, unpublished data, 2010).

Activity patterns with higher activity in the warmer part of the day and/or lower activity in the colder part are surprisingly common in so far studied rodents with dominant subterranean activity (see Table 4 for data from the available literature). The burrow temperatures given in the corresponding studies (including the present one) are usually well below the lower critical temperature of the thermoneutral zone of the studied animals (Table 4), which means that activities at these temperatures require an increased energy expenditure, and thus the most plausible explanation for the temperature-related activity patterns seems to be energetic savings on thermoregulation (cf. Benedix, 1994). Comparable laboratory data on strictly subterranean rodents kept under artificial temperature cycles are, to our knowledge, limited to *S. ehrenbergi*, who tended to be more active in the daily period when the ambient temperature was lower (23.9 °C; Goldman et al., 1997). Pohl (1998) published interesting data on 2 less fossorial rodents kept under artificial temperature cycles. While the high temperature-adapted antelope ground squirrel *Ammospermophilus leucurus* performed wheel running activity mainly during the lower temperature part of the day, in the cold temperature-adapted Syrian hamster *Mesocricetus auratus*, the wheel running primarily coincided with the warmer part of the cycle. Another rodent, the pacific pocket mouse *Perognathus longimembris*, displayed arousal from torpor just before a period of elevated temperature (Lindberg and Hayden, 1974). These studies illustrate that entrainment of activity rhythms in rodents by a temperature

Table 4. Relationship of activity patterns of free-living subterranean rodents with temperature fluctuation and selected physiological characteristics of studied species connected to their thermoregulatory capabilities.

		Physiological Parameters			Activity-Related Observations				
Species	**Source[a]**	**Body Mass, g**	**Conductance,[b] μL O_2 g^{-1} h^{-1} $°C^{-1}$**	**TNZ, °C**	**Source[a]**	**Season**	**Mean Daily Range of Burrow Temperature, °C**	***n***	**Activity Pattern Characteristic**
Thomomys bottae	a	143	62	28-30	h	Summer	22-26	8	Peak coincided with the maximum burrow temperature.
Geomys bursarius	b	197	100-190	30-32.5	i	Summer	21-24	11	Avoiding both temperature extremes.
Spalax ehrenbergi	c	124	142?	28.4-?	j	Winter	13-15	8	Peak between the times of maximum surface and maximum burrow temperature.
						Summer	31-34	3	Peak coincided with the minimum burrow temperature.
Spalax galili	c	104	124?	26.4-?	k	Winter	6-8	20	Peak between the times of maximum surface and maximum burrow temperature.
Heliophobius argenteocinereus	d	223	77	25-33	l	Cold dry season	17-19	10	Positively correlated with the burrow temperature.
						Beginning of hot dry season	18- 22	9	No correlation with the burrow temperature.
					m	Peak hot dry season	24-26	7	Peak close to the time of the minimum burrow temperature.
Fukomys damarensis	e	125	85	27-31	n	Summer	34-38	Group of 5	No correlation with the burrow temperature.
Fukomys mechowii	f	272	90	29-30	o	Cold dry season	18-20	Group of 5	No correlation with the burrow temperature.
						Cold dry season	18-20	1 disperser	Peak coincided with the maximum burrow temperature.
Fukomys anselli	f; g	77; 82	120; 144	28-32; 30-32.5	p	Cold dry season	18-26	17 (of 5 groups)	Positively correlated with the burrow temperature.

Burrow temperatures and soil temperatures measured roughly at depth of most foraging burrows are not differentiated.

[a] Sources: a = Vleck, 1979; b = Bradley and Yousef, 1975; c = Nevo and Shkolnik, 1974, and Haim and Izhaki, 1993; d = Zelová et al., 2007; e = Lovegrove, 1986; f = Bennett at al., 1994; g = Marhold and Nagel, 1995; h = Gettinger, 1984; i = Benedix, 1994; j = Rado et al., 1993; k = Hadid et al., 2013, and J. Šklíba and M. Lövy, unpublished data, 2012; l = Šklíba et al., 2007; m = J. Šklíba and M. Lövy, unpublished data; n = Lovegrove, 1988; o = Lövy et al., 2013; p = present study.

[b] Conductance (measure of heat losses) below the lower critical temperature.

cycle may involve both high and low temperature preference/avoidance and may not be limited to only heterothermic rodents.

Fukomys anselli has very high thermoregulatory requirements to maintain stable body temperature below the lower critical temperature, as can be derived from relatively high values of minimal conductance when compared with larger bathyergids and especially with subterranean rodents from other families (see Table 4 and corresponding citations).

This is mostly a consequence of its low body mass and relatively large body surface (similar values of conductance were detected for some other small-bodied mole-rats; see Zelová et al., 2007). Because the mean range of daily burrow temperatures during our radio-tracking study was 18 to 26 °C, the mole-rats had to deal with temperatures below their lower critical temperature (28 °C; Bennett et al., 1994) with the result that the lower the burrow temperatures were, the more energy the animals spent on thermoregulation. This means that at 26 °C and 18 °C, the mole-rats have to increase their energy expenditure by 38% and 190%, respectively, compared with their resting metabolic rate (which is 0.63 ml O_2 g^{-1} h^{-1}; Bennett et al., 1994), just to maintain thermoregulation. We will attain even higher metabolic increases if we consider the lower critical temperature to be 30 °C as reported by Marhold and Nagel (1995). Observed outside-nest activities synchronized with high burrow temperatures are thus favorable from the energetic point of view.

Since the high relative humidity in the burrows of subterranean rodents complicates evaporative cooling, it might be necessary for these animals to be better physiologically adapted to cope with high burrow temperatures than with low temperatures, as they need to spend some time per day digging and pushing soil in surface tunnels. (Subterranean rodents were rarely reported to build food caches large enough to span extended periods of time such as the hot dry season in tropical Africa; Šklíba et al., 2011, and references therein.) The lack of an effective cooling mechanism makes higher temperatures potentially more fatal than lower ones, which can be coped with by torpor (a mechanism also known in *F. anselli*, Marhold and Nagel, 1995), thermogenesis, and various behavioral mechanisms such as building nests with insulating nest bedding and, in social species, huddling in a communal nest. Behavioral mechanisms that reduce overheating, such as resting breaks during digging, cooling in deeper tunnels, and heat dissipation by increasing physical contact with the substrate (cf. Marhold and Nagel, 1995; Zelová et al., 2007), may not be sufficient if not coupled with high conductance above the upper critical temperature. Because conductances below the lower critical temperature and above the upper critical temperature are interconnected (both are influenced, e.g., by fur quality), better physiological adaptation to low temperatures would result in worse adaptation to high temperatures, which are more likely to be fatal in subterranean ecotope. We may therefore expect that daily activity patterns in small bathyergids will probably be more adjusted to avoid the coldest daily temperatures. Published data support this assumption (Table 4), although activity data from the hottest periods of the year are lacking.

The herein described activity patterns in Ansell's mole-rats come from the cold period of the year. Whereas the temperature inside the nests is probably constant year-round, because of depth, communal nesting, and a large amount of bedding (Šklíba et al., 2012), seasonal variation of temperature at shallower depths can be prominent (e.g., Šumbera et al., 2004). Seasonal changes in daily activity pattern of mole-rats would thus be a very effective tool for saving energy on thermoregulation while reducing the risk of overheating. This kind of seasonal change in the activity pattern was described in *S. ehrenbergi* (Rado et al., 1993), and indications of it were also found in the solitary bathyergid *H. argenteocinereus* (Šklíba et al., 2007; J. Šklíba and M. Lövy, unpublished data in Table 4). It is also relatively common in other rodents, such as fossorial *S. cyanus* (Rezende et al., 2003) and less fossorial hairy-tailed Bolo mouse *Necromys lasiurus* (Vieira et al., 2010), Cabrera vole *Microtus cabrerae* (Pita et al., 2011), and the ice rat *Otomys sloggetti* (Hinze and Pillay, 2006). There is a possibility that the activity pattern herein described in *Fukomys anselli* is also transient, confined to the colder part of the year.

Daily temperature fluctuations are substantially buffered, even in top layers of the soil (Bennett et al., 1988), so it might be surprising that even small temperature differences would still affect activity patterns of subterranean rodents. Gettinger (1984) found that peak activity of the pocket gopher *Thomomys bottae* occurred when mean burrow temperature was the highest (≈26 °C), but he considered it unlikely that rest periods were timed to avoid lower temperatures since burrow temperatures during these time periods were "not substantially different (≈22 °C)." Nevertheless, a similarly small daily range of burrow temperatures (4 °C) was later detected in another species of the pocket gopher, *Geomys bursarius*, whose daily activity was even bimodal, avoiding both temperature extremes (Benedix, 1994). Many other field studies of subterranean rodents revealed signs of temperature-affected daily activity patterns under very small daily temperature changes (Table 4). During our study, the mean amplitude of temperature changes at depth of foraging tunnels was relatively large (8 °C) where the surface soil was exposed to the sun but was reduced to only 1.8 °C under dense vegetation (nevertheless, more exposed places dominated in the study area). To estimate the minimal daily temperature difference that would elicit behavioral response, further laboratory experiments are necessary. Captive and usually ad libitum fed laboratory animals are not likely to display as fine-tuned energy-saving behavioral strategies as might be expected under natural conditions, but useful information can be found even in studies of circadian rhythms and their maintenance on account of temperature changes. Goldman et al. (1997)

described a case of 1 captive blind mole-rat who coordinated locomotory activity with a temperature phase even after the temperature differential was decreased to 1.1 °C. Similar temperature amplitude (1.5 °C) was reported to entrain circadian rhythms in *P. longimembris* (Lindberg and Hayden, 1974). In the light of these anecdotic observations, the temperature amplitude between 1.8 and 8 °C detected in the present study seems to be most probably large enough to affect the behavior of the Ansell's mole-rat.

A question remains as to whether the daily activity pattern detected in this species under natural conditions indicates entrainment to a temperature change as a nonphotic zeitgeber or represents masking. This is a challenge for future laboratory studies, but one point should be taken into account—the communal nest of social mole-rats is probably temperature-stable (Šklíba et al., 2012; Šumbera et al., 2012). This means that a change of ambient temperature would hardly trigger activity of an individual that is resting inside. The entrainment of circadian rhythms by temperature changes in the tunnels near the surface thus seems to be more plausible explanation for the observed pattern than masking. Impact of temperature changes on resetting circadian clock was also proposed in *Spalax* (Oster et al., 2002).

In the present study, the group affiliation explained a larger part of the interindividual variability in the activity pattern than did sex × reproductive status and body mass. Members of the same family group thus tended to be more similar in their activity patterns, as visible even in their actograms: For example, group P01 started to be active slightly earlier than group P04 (Fig. 1). Therefore, we would assume that individuals within a group are rather synchronized in their outside-nest activity. Surprisingly, a significant synchronization of the individuals' outside-nest activity was detected only in 2 of the 5 family groups. We would need more frequent activity sampling to address this problem in greater detail. Only an indistinctive synchrony of sleep and wakefulness was detected in captive family groups of *Heterocephalus glaber*, which as a whole did not perform any activity rhythms under either of the applied light regimes (Davis-Walton and Sherman 1994). Members of captive family group of *Cryptomys hottentotus* under constant dark performed asynchronously in their activity cycles (Bennett, 1992).

The activity pattern of *F. anselli* differs markedly from that described in *F. damarensis* (Lovegrove 1988) and *F. mechowii* (Lövy et al., 2013). The latter 2 species were active in multiple short bouts per day, which were only slightly more concentrated into the night hours in *F. mechowii*. Lövy et al. (2013) proposed that the high number of short activity bouts per day could be a byproduct of the use of the communal nest by many individuals, and therefore more frequent disturbance by other family members, or a consequence of cooperation in work tasks. Similar influence of social contact with other group members on the individuals' activity patterns, which could be viewed as social masking, was illustrated in captive *H. glaber* by Riccio and Goldman (2000). Whereas most of the singly-housed individuals performed clear light-entrained or free-running activity rhythms, individuals monitored while they were housed in a group were mostly arrhythmic. In our study the distinct daily activity pattern performed by Ansell's mole-rat, despite using communal nest, would signify that the effect of the external determinant of activity (i.e., most probably the daily temperature fluctuations) was stronger than in previous radio-telemetry studies of social African mole-rats (Lovegrove, 1988; Lövy et al., 2013). This is not surprising since this species has low thermoregulatory capabilities at low temperatures (see Table 4) and a burrow system mostly void of deep primary burrows (Šklíba et al., 2012), where the temperature fluctuations would be less prominent. We can therefore conclude that social cues in communally nesting mole-rats can disrupt temperature-related daily activity patterns, but probably only if the additional cost of thermoregulation is not too high. Anecdotic support for this is provided by the activity pattern of a dispersing female *F. mechowii* radio-tracked by Lövy et al. (2013). This solitarily living individual displayed a clear activity peak at a time of maximum temperature at depth of the foraging tunnels, unlike the rest of individuals under study, which lived in a family group.

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CONFLICT OF INTEREST STATEMENT

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Chapter 5

Behavioural tests reveal severe visual deficits in the strictly subterranean African mole-rats (Bathyergidae) but efficient vision in the fossorial rodent coruro (*Spalacopus cyanus*, Octodontidae)

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In prep.

Behavioural tests reveal severe visual deficits in the strictly subterranean African mole-rats (Bathyergidae) but efficient vision in the fossorial rodent coruro (*Spalacopus cyanus*, Octodontidae)

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Abstract

Vision has long been considered purposeless in the dark underground ecotope. However, recent anatomical studies revealed an unexpected diversity of ocular and retinal features and various degrees of development of the visual system in mammals with dominant subterranean activity, and have suggested retention of basic visual capabilities even in some strictly subterranean mammals such as the African mole-rats. Behavioural tests assessing image-forming vision have not yet been conducted in subterranean mammals. Here, we investigated the visual capacities in three species of the African mole-rats, namely in the giant mole-rat *Fukomys mechowii*, the Mashona mole-rat *Fukomys darlingi* and the silvery mole-rat *Heliophobius argenteocinereus*, in the fossorial coruro *Spalacopus cyanus* and the inbred C57L/J mouse. The behavioural assays performed in this study revealed severe visual deficits in all three species of mole-rats. The absence of the visual placing reflex suggested impairment of either image-forming vision or visuomotor integration. The random choice between the shallow and the deep side of the visual cliff clearly demonstrated inability of mole-rats to perceive depth. The nesting assay did not yield conclusive evidence regarding the capacity for visually guided spatial orientation. In contrast, both the coruro and the mouse exhibited a clear placing reaction and preferred the shallow side of the visual cliff, implying functional image-forming vision. Thus, the behavioural data gathered in this study show that vision is seriously compromised in the strictly subterranean, congenitally

microphthalmic African mole-rats but efficient (i.e., comparable to that of surface-dwelling rodents) in the fossorial coruro.

Introduction

Vision provides unique information about spatial structure of environment and it has probably been a key selective advantage enhancing survival throughout animal evolution (Dusenbery 1992, Land & Nilsson 2002). Not surprisingly, many species have drastically modified their visual systems in response to light conditions and visual challenges of the ecotope they are adapted to. In the context of visual ecology, the subterranean ecotope is arguably one of the most extreme sensory environments, being deprived of light and all visual cues available above ground. Among mammals, as many as 300 species adapted to the underground ecotope (reviewed in Nevo 1999, Lacey, Patton & Cameron, 2000). It provides shelter from predation and climatic fluctuations as well as food sources unavailable for surface dwellers. On the other hand, the underground ecotope imposes special sensory requirements on its inhabitants. Strictly subterranean mammals typically have a reduced visual system (for review, see Němec et al. 2007) but extraordinary tactile (for review, see Kimchi & Terkel 2004, Park et al. 2007) and, in some species, also magnetosensory capacities (for review, see Moritz et al. 2007). Indeed, strictly subterranean, congenitally microphthalmic mammals have long been considered fully blind (Eloff 1951, 1958, Burda et al. 1990a). However, more recent neuroanatomical studies revealed unexpected diversity of ocular and retinal features and various degrees of development of the visual system in mammals with dominant subterranean activity, suggesting different visual capabilities and adaptations in different species (for review, see Peichl 2005, Němec et al. 2007).

The African mole-rats (Bathyergidae, Rodentia) belong to strictly subterranean rodents, which rarely emerge aboveground. They construct and inhabit extensive burrow systems isolated from the aboveground environment by mounds of soil and feed almost exclusively on geophytes, i.e., plants with subterranean storage organs (e.g., Bennett & Faulkes 2000). In most species, the above ground activities appear to be restricted to rare, non-recurring events such as natal dispersal, emigration after depleting of food resources or flooding. In the silvery mole-rat, more regular surface activities such as searching for mates during mating season have been reported (Patzenhauerová et al. 2010). Despite their strictly subterranean mode of life, the African mole-rats possess small, superficially located and structurally normal eyes, which have retained image-forming properties: eyelids, clear cornea, lens, and vitreous, iris with a pupillary aperture and a well-developed retina lining the back of the eye (Cernuda-Cernuda et al. 2003, Peichl et al. 2004). Nevertheless, the image-forming vision may be compromised due to spherical aberration associated with small, round lenses (Hetling et al. 2005), very low visual acuity (Němec et al. 2008), and, at least in some species, also due to enlarged, highly pigmented ciliary body (Nikitina et al. 2014) and disorganized lens (Nikitina et al. 2004) and retinal architecture (Mills &

Catania 2004). A reduced, non-laminated dorsal lateral geniculate nucleus (Negroni et al. 2003, Němec et al. 2004, Crish et al. 2006) and an extremely small primary visual cortex (Němec et al. 2008, Molnár et al. 2014) also imply a minor importance of image-forming vision. Finally, severely reduced superficial visual layers of the superior colliculus and a vestigial accessory optic system (Němec et al 2004) indicate that the bathyergid mole-rats are poorly equipped for the detection and orientation towards objects in the visual field and incapable of stabilizing the image on the retina during head and body movements (for further discussion, see Němec et al. 2008).

The aforementioned regressive features render above-ground visually guided navigation, foraging, seeking for conspecifics, and predator avoidance ineffective if not impossible. Therefore, it has been suggested that the main function of residual, low acuity vision is to localize breaches in the burrow systems that let in light and thereby act as an early warning system helping subterranean mammals to avoid predators (Hetling et al., 2005, Wegner et al. 2006, Němec et al. 2007, 2008). The fact, that the eye of bathyergid mole-rats seems to be adapted to bright-light rather than low-light conditions, is in line with this hypothesis. Small lenses that collect light rather ineffectively (Němec et al. 2008), high cone proportions (Peichl et al. 2004) and rod nuclei with conventional chromatin architecture (unpublished data, cf. Solovei et al. 2009) are characteristic of diurnal mammals and are not expected in animals adapted to subterranean darkness. More direct support for the hypothesis comes from laboratory experiments: plugging of illuminated tunnels has been reported in two bathyergid species (Kott et al. 2010). Moreover, recent study (Kott et al. 2014) analyzing photic conditions in damaged tunnels of Ansell´s mole-rat (*Fukomys anselli*) confirms that amount of light which penetrates to a damaged burrow system during a day and also a moonlit night suffice to inform an underground dweller about the site of potentially increased predation risk.

For now, most of what is known about vision in bathyergid mole-rats has been inferred from anatomical evidence. Behavioural, physiological and field studies mainly focused on mechanisms involved in controlling their circadian and circannual biological rhythms. All species investigated thus far display locomotor activity rhythms loosely entrained to light under experimental conditions (Lovegrove & Papenfus 1995, Riccio & Goldman 2000, Oosthuizen et al. 2003, Hart et al. 2004, Vasicek et al. 2005, Schottner et al. 2006, de Vries et al. 2008) and circadian rhythms of melatonin secretion (Richter et al. 2003, Gutjahr et al. 2004, Vasicek et al. 2005) and body temperature (Lovegrove & Muir 1996, Riccio & Goldman 2000). Nevertheless, recent field studies have suggested that burrow temperature can work as zeitgeber of circadian activity under natural conditions (Šklíba et al. 2007, 2014). Actual visual capacities have received only scant attention. Ability to discriminate between light and dark has been demonstrated in five mole-rat species (Wegner et al. 2006, Kott et al. 2010) and capacity to perceive short to medium-wavelength light at photopic light levels in two species (Kott et al. 2010). To our knowledge, no single behavioural test has been conducted to assess image-forming vision in subterranean mammals.

In this study, we investigated the visual capacities in rodents that differ in degree of confinement to subterranean mode of life. Three species of the African mole-rats (namely, the giant mole-rat *Fukomys mechowii*, the Mashona mole-rat *Fukomys darlingi* and the silvery mole-rat *Heliophobius argenteocinereus*), the coruro *Spalacopus cyanus* and the inbred C57L/J mouse were chosen as representatives of strictly subterranean, fossorial, and epigeic rodents, respectively. The coruro inhabit extensive systems of self-constructed tunnels and harvesting of geophytes through tunnelling appears to be their primary mode of foraging, albeit there is also evidence of foraging on vegetation around the tunnel opening (Reig 1970, Contreas et al. 1993, Torres-Murra & Contreas 1998, Begall & Gallardo 2000, Urejolla et al. 2005). It possesses large eyes with diameter of nearly 6 mm (Peichl et al. 2005) and unreduced visual system (Cooper et al. 1993a,b, Němec et al. 2007). The mouse was also used for essay validation, because it is a typical model for such experiments. The C57L/J inbred mouse was selected because no naturally occurring mutants that manifest retinal degeneration were reported in this strain (cf., Chang et al 2002).

Three experimental paradigms were utilized. Firstly, we tested vision and visuomotor integration by assessing presence/absence of the visual placing reflex (forepaw extension) when animals were lowered toward a visible surface (Irvin 1968). Secondly, capability of visual depth or distance perception was tested using the visual cliff (Walk et al. 1957, Gibson & Walk 1960, Walk & Gibson 1961). Finally, to find out whether bathyergid mole-rats are capable of visually guided spatial orientation, we examined effect of salient visual cue on orientation of the giant mole-rat in the circular arena. The results of our study indicate severe visual deficits in the African mole-rats but efficient vision in the coruro.

Material and Methods

Ethical Note

Animal husbandry and all experimental procedures complied with the European Community regulations on the care and use of experimental animals, and were approved by the Intuitional Animal Care and Use Committee of University of South Bohemia and by Ministry of Education, Youth and Sports of the Czech Republic (Permission No. 12924/2007-30).

Study animals

The silvery mole-rat (*Heliophobius argenteocinereus*, Peters 1846) live in southern Kenya, Tanzania, Malawi, southeast D. R. Congo, eastern Zambia, and northern Mozambique; the giant mole-rat (*Fukomys mechowii*, Peters 1881) inhabits northern Zambia, south D. R. Congo, and Angola; the Mashona mole-rat (*Fukomys darlingi*, Thomas 1895) inhabits central Zimbabwe, Mozambique and southern Malawi. Coruro (*Spalacopus cyanus*, Thomas 1925) is endemic to Chile.

The silvery mole-rats were caught in Malawi in Mpalanganga estate, Zomba (15° 27´S, 35° 15´E), Zomba plateau (15° 20´S, 35° 16´E), and Mulanje - Chipoka (16° 02´S, 35° 30´E) in 2005 and in Zambia (Kacholola, 14° 45´S, 30° 35´E) in 2010. The Mashona mole-rats were caught in 2005 in Malawi (Nsanje, 16º55´S, 35º16´E). Some of the giant-mole rats were caught in 1999 in Ndola in Zambia; the rest were born in captivity. Coruros were obtained from breeding colonies in the University of Duissburg-Essen, Germany. The inbred C57L/J mice originating from the Jackson Laboratory (Bar Harbor, Maine, USA) were obtained from breeding stock in the University of South Bohemia.

The mole-rats and coruros were kept in an animal room with temperature 25±1°C and a 12/12 light regime (light on 7.00 -19.00) at the University of South Bohemia. The silvery mole-rats were housed individually in plexiglass mazes, families of the Mashona mole-rats, giant mole-rats and coruros in terrariums. Laboratory mice were kept under the same light conditions and temperature 21±1°C in a separate breeding room in plastic boxes Velaz TIII (430×275×150 mm). The animals were fed ad libitum with carrots, potatoes, lettuce, apples, and rodent pellets.

Experiment 1 -Visual placing reflex

Animals were observed for the visual placing reflex (forepaw extension) when lowered toward a visible surface (Irvin 1968). Each tested individual was lifted by the base of tail to a height of approximately 20 cm and lowered towards a surface within 0.5-1 second, decelerating as the surface had been approached. Presence or absence of the reflex and the distance of the animal´s snout from the surface, at which the reflex was initiated, were scored. In a first set of trials, we tested three species of African mole-rats – *F. mechowii* (N=30), *F. darlingi* (N=30) and *H. argenteocinereus* (N=29), coruro (N=23) and laboratory mouse (N=26) and used a grid pattern as a surface (3×3 cm, Fig. 1A). Because the African mole-rats have extremely low visual acuity (Němec et al. 2008) and might therefore have difficulties to see lines forming the grid, we used blue-white checkered pattern as a surface (3×3 cm, Fig. 1B) in a second set of trials. Only *F. mechowii* (N=22), *F. darlingi* (N=22) and *H. argenteocinereus* (N=13) were used in this second stage of the experiment. Each animal was tested only once in each experimental design.

Experiment 2 -Visual cliff

The visual cliff apparatus used in our experiment was modified from original Model II described by Walk & Gibson (1961). The apparatus comprised of a central elevated platform laid across a terrarium (65×43×40 cm) placed on a 50 cm high glass table (Fig. 1C). Because rodents are capable of using tactile sensation mediated by whiskers for depth perception in this experimental paradigm (Walk & Gibsson 1961, Schiffman et al. 1970), care was taken to use platform high enough to prevent contact of whiskers with the bottom of the terrarium (size of the platform: 43×5×5 cm for *S. cyanus* and *M. musculus*; 43×15×8 cm for mole-rats). A 3×3 cm checkerboard pattern was used to create visual illusion of a cliff. It covered the elevated platform and was laid directly beneath the bottom of the terrarium on “a shallow side” and upon the floor on “a deep side”. The apparatus was illuminated by fluorescent light tubes mounted 150 cm above the terrarium, centred to secure homogenous illumination and adjusted to minimize reflections. The light intensity at the bottom of the terrarium was about 6 µmol photons s^{-1} m^{-2}, as measured by a data logger (Minikon QT, EMS, Czech Republic). This light intensity is clearly in the photopic range, where cone signals dominate and rods contribute little, if anything, to the ganglion cell response (for details, see Kott et al., 2010, 2014).

A red-white checkerboard pattern was used in a pilot experiment. However, the African mole-rats need not see red light at photopic light levels, because their cones express dominantly a short-wave-sensitive opsin (see Introduction). Indeed, a lack of photoavoidance response to red light was reported in the silvery mole-rat and the giant mole-rat (Kott et al., 2010). Therefore, blue-white checkerboard pattern was used in follow-up experiments. Each animal was tested only once in each experiment. Between trials, the whole apparatus was thoroughly cleaned with ethanol.

Video surveillance was used to monitor a side choice and the total time needed to descend from the elevated platform. Tested animals were individually placed on the platform and their behaviour was recorded using an overhead video camera TAYAMA C4702-01A1 coupled to computer via Chateau RT4 video grabber (Westport, U.S.A.). Each experimental animal was recorded until it descended or for maximum of ten minutes. Species typical side choices were analyzed using chi-square tests, interspecific differences in descent latencies using one-way analysis of variance (ANOVA) followed by Tukey post hoc tests. Latency values (s) were log-transformed before the subsequent statistical analyses. A 95% confidence level ($P < 0.05$) was used to judge statistical significance.

Experiment 3 - Cue card

The behavioural assay designed to assess magnetic compass orientation in mole-rats (Burda et al. 1990b) was modified to test their capacity for visually guided spatial orientation. Several species of the Ansell's mole-rats exhibit a spontaneous drive to build nests in a particular sector of a circular arena using magnetic field azimuth as the primary orientation cue (Burda et al. 1990b, Oliveriusová et al. 2012). We have tested whether placing of familiar, salient visual cue will affect mole-rat directional preference in the arena. Only the giant mole-rat was tested in this time-consuming experiment. This species was selected because it has eye and optic nerve without obvious degenerations as well as the highest total number of optic nerve fibres and the highest visual acuity of three bathyergid species studied (Němec et al. 2008, unpublished data). Moreover, it exhibits a spontaneous motivation to build nests in a circular arena (Oliveriusová et al. 2012).

Four family groups (five to seven members each) of the giant mole-rats were tested. Three weeks before beginning of the experiment, all the groups were provided with the cue card (sheet of white paper, 49.5×40.5 cm) placed against the outer wall of their home-terrariums in a close vicinity of their nests. A purpose of this pre-training was to give mole-rat chance to associate the cue card with a nest position. After pre-training period, the animals were released in a circular arena (81cm diameter and 41 cm high; made of dark brown plastic impervious to light) equipped with inner transparent plexiglass cylinder (80 cm diameter and 39 cm high), Fig.1D. The cue card subtending angle of 70° was inserted between outer and inner walls of the arena to prevent its destruction by mole-rats tested. The azimuthal position of the cue card was changed in each trial in a pseudorandom fashion. The arena was covered by a plastic diffuser and illuminated from above by means of a centred 75 W full spectrum light TRILITE III (Ades, Czech Republic); the light intensity at the bottom of the arena was about 15 µmol photons s^{-1} m^{-2}. The arena was filled with a thin layer of horticultural peat as litter, 20 scattered strips of a filter paper as nest material, and three randomly distributed pieces of carrots as food. Animals were allowed to explore the arena for two hours, immediately afterwards the nest position was recorded and taken as a proxy for assessing directional choice. Each group was tested in 20 two hours long trials with the cue card and groups 2–4 subsequently in 20 additional trials without cue card to assess effect of the cue card on mole-rat directional preference. Trials, in which mole-rats did not build a nest within two hours, were excluded from statistical analyses. Between trials, the horticultural peat, nest material and food pieces were removed from the arena and discarded, and the arena was thoroughly cleaned with 70% ethanol.

Directional responses were analysed using circular statistics (Batschelet, 1981). The Rayleigh test was used to assess significant deviations from a random distribution of bearings. Because the same experiment was performed in four family groups, the Sidak correction was used to counteract the problem of multiple testing. The Watson-Williams F-test was used to compare the mean bearings between tests performed with and without cue card; the Mardia-Watson-Wheeler test was used for determining whether two distributions were identical. All tests were calculated with Oriana 4.01 (Kovach Computing Services).

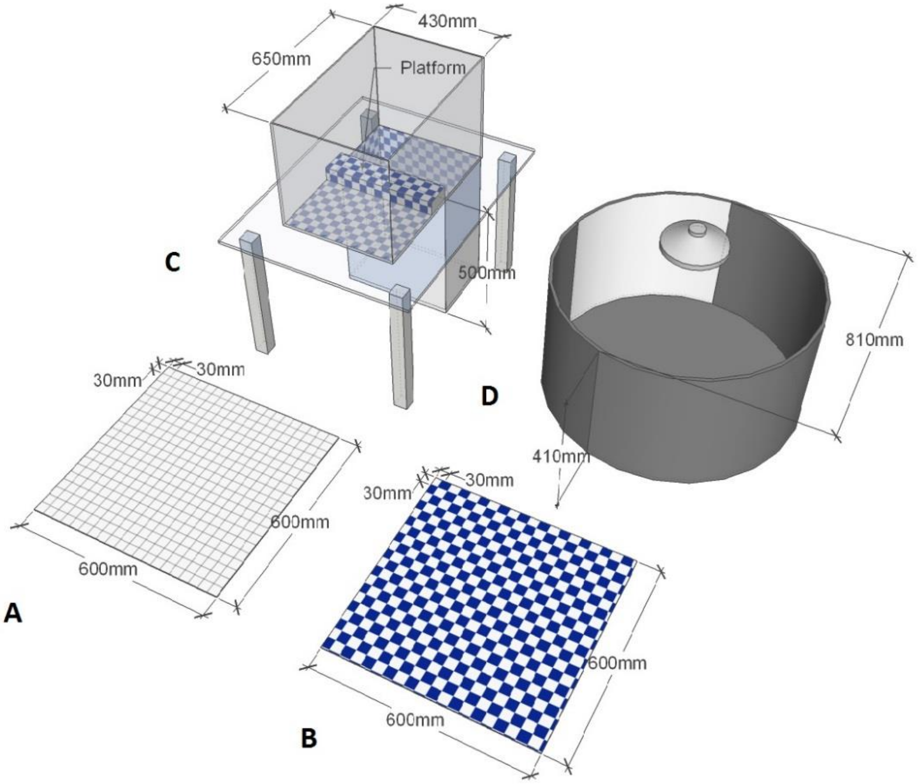


Figure 1. Experimental apparatus schema: (A) Visual placig reflex – grid, (B) Visual Placig Reflex – blue-white checkered pattern, (C) Visual Cliff, (D) Cue Card Test

Results

Experiment 1 – Visual placing reflex

The visual placing reflex was absent in all bathyergid species examined (Fig. 2). Indeed, not a single mole-rat exhibited overt behavioural reaction when lowered toward the visible surface, no matter whether grid or blue-white checkered pattern was used as a surface. Mice and coruros, by contrast, showed a clear placing reaction (Fig. 2). Mice typically extended forelimbs at a distance of ~6 cm from the surface. In fossorial, short-limbed coruros, the reflex occurred at the same height but was restricted to stretching forepaw fingers.

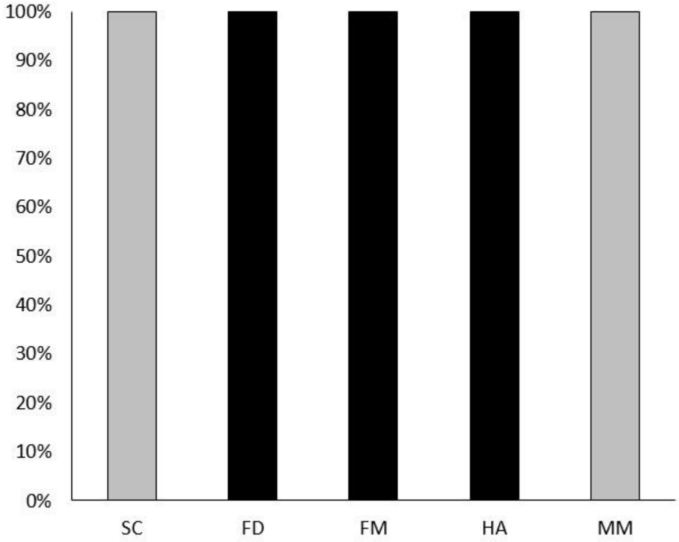


Figure 2. Visual placing reflex (VPR) in *Spalacopus cyanus* (SC), *Fukomys darlingi* (FD), *F. mechowii* (FM), *Heliophobius argenteocinereus* (HA) and *Mus musculus* (MM). Black bars represent percentage of absence and grey bars of presence VPR.

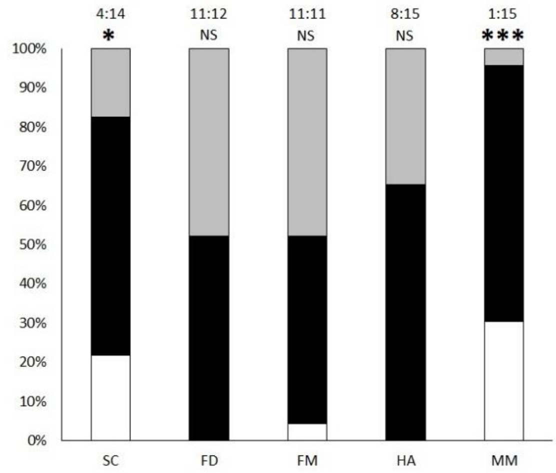
Experiment 2 – Visual cliff

All three mole-rat species showed a random choice between the shallow and the deep side of the visual cliff (blue-white checkerboard pattern: *F. darlingi*, χ^2=0.04, P=0.83, N=23; *F. mechowii*, χ^2=0.0, P=1, N=23; *H. argenteocinereus*, χ^2=2.13, P=0.14, N=23, Fig. 3A; red-white checkerboard pattern: *F. darlingi*, χ^2=0.0, P=1, N=26, Fig. 3B). They typically descended from the central elevated platform quickly (Fig. 3C) and exhibited no signs of vigilance. Indeed, out of 69 mole-rats tested in 95 trials, only one individual of the giant mole-rat did not descent from the platform in one trial.

In contrast, both the coruros and laboratory mice significantly preferred the shallow side of the cliff (blue-white checkerboard pattern: *S. cyanus*, χ^2=5.56, P=0.018 N=23; *M. musculus*, χ^2=12.25, P<0.001, N=23, Fig. 3A; red-white checkerboard pattern: *S. cyanus*, χ^2=10.89, P<0.001, N=23; *M. musculus*, χ^2=15.21, P<0.001, N=26, Fig. 3B). Although coruros often made decision quickly (Fig. 3C), they did not descend from the elevated platform in ~ 22 % of cases (in 10 out of 46 trials). Mice were the most vigilant; it took them longer to make decision (Fig. 3C) and they did not descend from the platform in ~ 30 % of cases (in 14 out of 49 trials).

Quantitative analysis of the experiment with blue-white checkerboard pattern confirmed significant interspecific differences in descent latencies (one way ANOVA, $F_{4,97}$ = 17.7, P < 0.001; Fig. 3C). Specifically, mice spent significantly more time on the central elevated platform than animals of any other species tested and the giant mole-rat spent more time on the platform than the Mashona mole-rat (Tukey post hoc test, P < 0.001for all pair wise comparisons).

A



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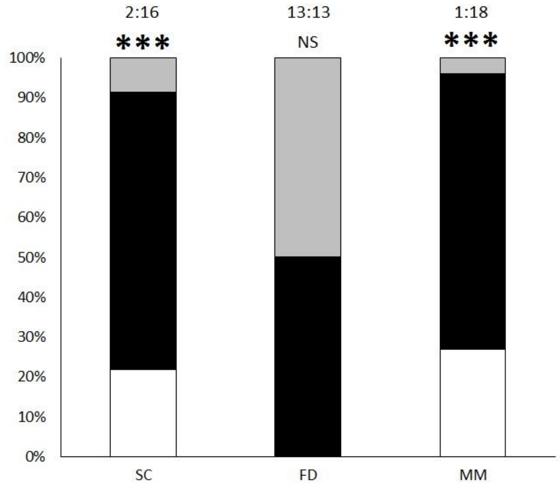

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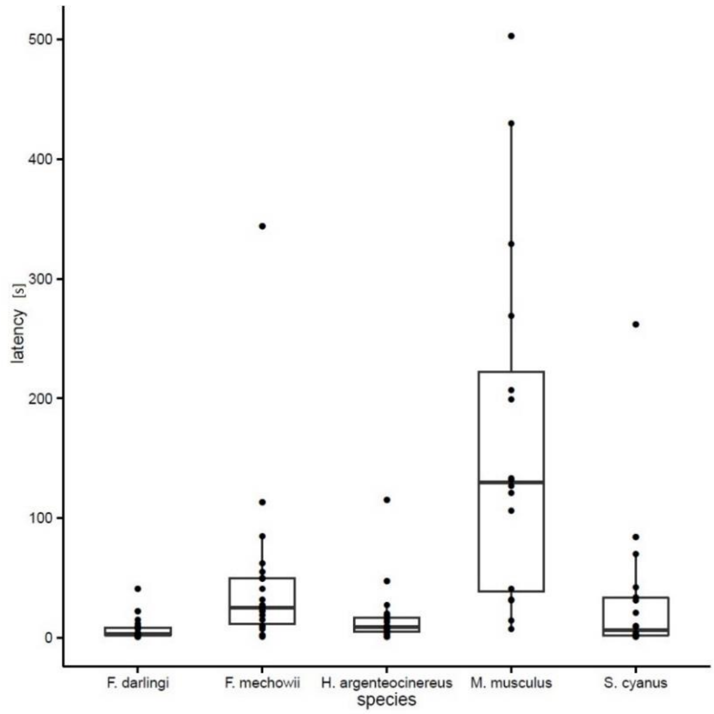

Figure 3. Depth perception in *Spalacopus cyanus* (SC)*, Fukomys darlingi* (FD), *F. mechowii* (FM), *Heliophobius argenteocinereus* (HA) and *Mus musculus* (MM). Black bars represent percentage of choices towards the shalow side, grey bars towards the deep side and white bars represent no descent in the visual cliff with blue-white (A) and red-white (B) checkerboard pattern. The statistical significance level of the response to the corresponding choice between deep and shallow side of the cliff are shown above each bar (***, P,0.001; *, P,0.05; NS, non significant).
(C) Interspecific differences in descent latencies (in seconds), horizontal lines denotes mean, box standard deviation (SD), vertical lines show 95 quartile and points individual observations.

Experiment 3 – Cue card

The giant mole-rats spontaneously gathered the nesting material and built a nest within two hours in the vast majority of trials. When tested with the cue card, family groups one and two exhibited a preference for building their nests in the southern sector of the arena, but only in group two the preference remained significant after Sidak correction for multiple testing (Rayleigh test: group 1, $\mu = 202° \pm 40°$ (mean vector orientation angle ± 95% confidence interval), $r = 0.425$ (mean vector length), $N = 20$, $P = 0.025$, $P = 0.162$ after Sidak correction; group 2, $\mu = 188° \pm 33°$, $r = 0.498$, $N = 20$, $P = 0.006$, $P = 0.041$ after Sidak correction; Fig. 4B); the topographic distribution of the nests in the arena was indistinguishable from random in groups three and four (Fig. 4B). When tested without the cue card, distribution of nests was random in groups three and four, group two tended to prefer southwest sector of the arena ($\mu = 225° \pm 44°$, $r = 0.391$, $N = 20$, $P = 0.045$, $P = 0.276$ after Sidak correction; Fig 4D).

Importantly, nest distributions were random when plotted relative to the cue card position in three out of four groups (Fig. 4C). In the group one, by contrast, nests were preferentially clustered in apposition to the cue card; but again significance did not withstand Sidak correction ($\mu = 14° \pm 41°$, $r = 0.417$, $N = 20$, $P = 0.029$, $P = 0.186$ after Sidak correction; Fig. 4C). Moreover, neither mean directional preference nor nest distribution was significantly different between tests performed with and without cue card (Watson-Williams F-test, $P > 0.05$ in all cases; Mardia-Watson-Wheeler test, $P > 0.05$ in all cases). Taken together, it can be concluded that azimuthal position of the cue card did not significantly affect orientation of the giant mole-rats.

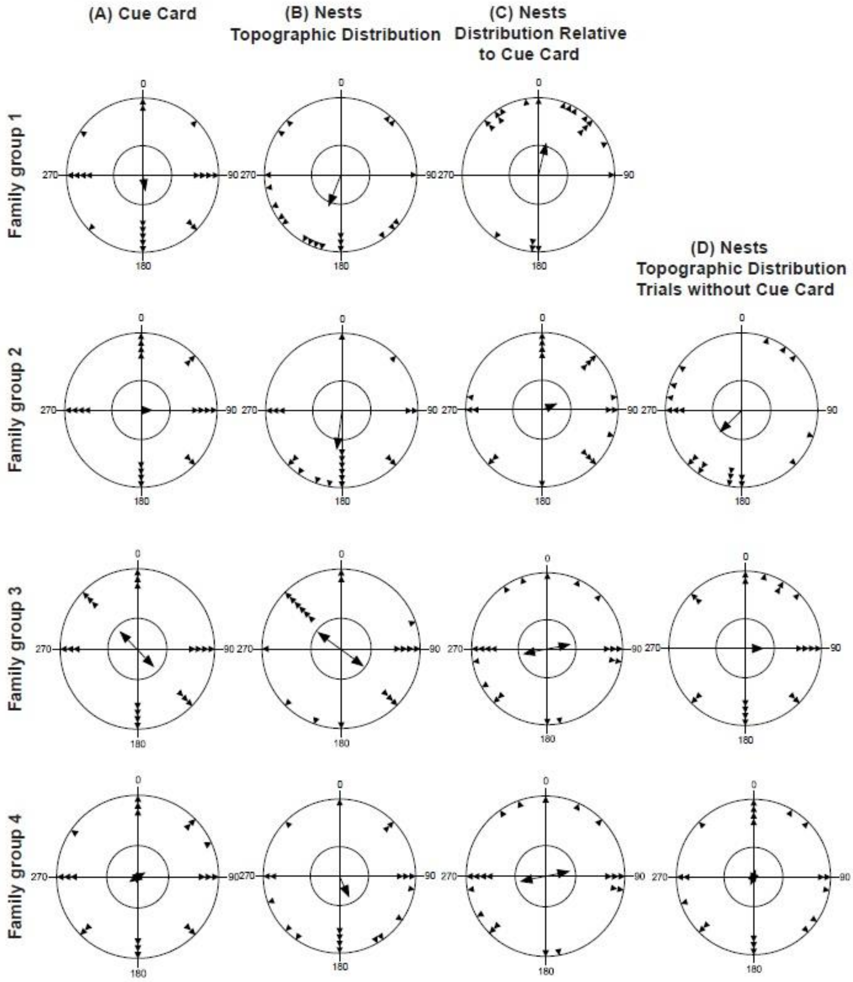

Figure 4**.** Orientation of the giant mole-rat *Fukomys mechowii* in a circular arena. (A) Distributions of cue cards. Each triangle represents the position of a cue card. (A–D) Distribution of nests in the arena. Each triangle represents the position of a nest built by a mole-rat family in one experimental trial. (B) Topographic distribution of nests in nests in the experimental trials conducted with the cue card. (C) Nests plotted relative to the azimuthal position of the cue card (standardized to 0°). (D) Topographic distribution of nests in the experimental

trials conducted without the cue card. Arrows indicate the mean vector for the distribution of the nests/cue cards; the length of the mean vector provides a measure of the degree of clustering in the distribution of the nests/cue cards. Double headed arrows indicate bimodal distribution of the nests/cue cards. The inner dashed circles mark the 5% significance border of the Rayleigh test; the arrows exceeding these circles indicate significant directional orientation.

Discussion

The behavioural assays performed in this study revealed severe visual deficits in three bathyergid species, the giant mole-rat *Fukomys mechowii*, the Mashona mole-rat *Fukomys darlingi* and the silvery mole-rat *Heliophobius argenteocinereus*. Absence of the visual placing reflex suggests impairment of either image-forming vision or visuomotor integration. A random choice between the shallow and the deep side of the visual cliff clearly demonstrates inability of mole-rats to perceive depth. The nesting assay did not yield conclusive evidence regarding capacity for visually guided spatial orientation (see below). In contrast, both the coruro *Spalacopus cyanus* and the inbred C57L/J mouse exhibited a clear placing reaction and preferred the shallow side of the visual cliff, implying an efficient image-forming vision. Thus, the behavioural data gathered in this study shows that vision is seriously compromised in strictly subterranean, congenitally microphthalmic African mole-rats but ‘normal’ (i.e., comparable with that of surface-dwelling rodents) in fossorial, large-eyed coruro, a finding consistent with anatomical evidence (for review, see Němec et al. 2007) and anecdotal observations (for review, see Burda et al. 1990a, Francescoli 2000).

Visual placing reflex is a simple test for observational assessment of vision, motor and spinocerebellar functions, which is commonly used as a part of a comprehensive behavioural assessment battery for analysis of mouse phenotype, often in context of pharmacological intervention or genetic manipulation (Irwin 1968, Rogers et al. 1997). Our observations provided clear-cut results: mice and coruros always exhibited an obvious placing reflex whereas mole-rats never shoved overt reaction when lowered toward the visible surface. The former observation evidences that the coruro is able to see grid pattern and that approaching visual stimulus efficiently elicits reflexive reaction, a strong indicative of a retained capacity for form and movement discrimination and visuomotor integration.

The situation is less conclusive in the African mole-rats. Because there is no a priory reason to presume a deficiency in motor and/or spinocerebellar functions in strictly subterranean mole-rats, we suppose that visual deficits underpin the absence of the visual placing reflex. At least four features of their visual system, which may synergically compromise image-forming vision, are to be considered. First, the eyes of bathyergid mole-rats are about 2 mm in diameter (Peichl et al. 2004, Němec et al. 2008). Small eye size limits the image size on the retina, resulting in poor image quality and visual acuity. Indeed, the upper limits of visual acuity estimated from peak of retinal ganglion cells and eye size, range between 0.3-0.5 cycles/degree (Němec et al. 2008). Moreover, the eyes are laterally positioned in the broad head. The broad snout obstructs the animal’s view in the forward direction, and the visual fields of the two eyes do not overlap in front of the animal (Němec et al. 2004). Second, despite structurally normal eyes (Cernuda-Cernuda et al. 2003, Peich et al. 2004, Hetling et al. 2005, Němec et al. 2008), visual processing might be severely impaired due to disorganized retinal architecture (Mills and Catania 2004). Specifically, the inner plexiform layer, where the processes of bipolar and amacrine cells contact the dendrites of ganglion cells, is very thin and considerably less regular than in more visual mammals. Furthermore, very little is known about the retinal ganglion cell types in bathyergid mole-rats. In well-studied mammals, including the mouse and the rat, about 15 types of retinal ganglion cells subserve different tasks in image analysis, e.g., form discrimination, movement detection, and colour vision and thus represent different visual processing channels (reviewed in Wässle 2004). It remains to be shown whether there are as many ganglion cell types and hence processing channels in bathyergid mole-rats. Third, the accessory optic system, which plays a seminal role in stabilizing the image on the retina during head and body movements, is vestigial. Via detection of whole visual field motion, it likely serves to signal self-motion and to coordinate eye and head movements in relation to the animal's own movement within the visual field (Simpson 1984, Cooper & Magnin 1986). Therefore, bathyergids are unable to stabilize image on the retina during movement and might also have difficulties to detect self-motion against a visual surface. Finally, functional coupling between visual stimuli and motor reflexes might be disrupted in strictly subterranean bathyergids due to relaxed natural selection cause by millions of years of underground existence.

The visual cliff is a classical test for visual depth or distance perception (Walk et al. 1957, Gibson & Walk 1960, Walk & Gibson 1961). Being based on inherent fear of heights, it involves no pretraining – animals spontaneously avoid descending over a vertical edge to a surface which appears to be far away. Indeed, avoidance of the deep side of the cliff have been reported in diverse animals, including rats, cats, sheep, pigs, goats, cows, chicken and turtles (Walk et al. 1957, Gibson & Walk 1960, Walk & Gibson 1961, Schiffman et al. 1970, Arnold et al. 2007). Here, we observed clear avoidance behaviour in surface-dwelling mouse and fossorial coruro, providing evidence of both depth perception and acute fear of heights in these rodents. The African mole-rats, by contrast, showed a random choice between the shallow and the deep side of the visual cliff. However, because of potentially disrupted image-forming vision (see above) and the absence of stereoscopic vision, the inability to perceive depth is to be expected in bathyergid mole-rats. Perception of depth (stereopsis) is based on binocular disparity, i.e., on small differences between images formed on the two retinae (Cumming & DeAngelis 2001). Since bathyergids possess no region of binocular viewing (see above), they cannot compare differences between the locations of matching features on the retinae and are therefore unable to judge the three-dimensional layout of the environment.

The laboratory mouse was more efficient in avoiding the deep side of the visual cliff and also more vigilant when placed on the central elevated platform of the visual cliff apparatus then the fossorial coruro. This difference can hardly be explained on the basis of anatomical organization of their visual systems. Both model species have normal eye morphology and well developed central visual system (Cooper et al. 1993a,b, Peichl et al. 2005, Chalupa & Williams 2008). In fact, the coruro possess larger eyes then the mouse: axial length of the eye is ~ 3.4 mm in the mouse and ~ 5.8 mm in the coruro (Remtulla & Hallett 1985, Peichl et al. 2005). We suggest that visual ecology of these models may offer more plausible explanation. Mice are not usually considered visual animals because they are nocturnal and relay principally on olfactory, auditory and tactile information to sense predators, food and conspecifics (Wong & Brown 2008). Nevertheless, the visual system of the mouse serves many functions, including tracking of moving targets, discerning spatial details of objects in the environment and spatial orientation (e.g., Stavnezer et al. 2002, Wong & Brown 2008). It should be noted here that although the laboratory settings provides an unnatural environment for the mouse, the visual system of laboratory mouse strains, except those with retinal degeneration, remains adapted natural conditions (Shupe et al. 2005). Importantly, mice inhabit various three-dimensional environments and are therefore regularly confronted with danger of a fall. In contrast, the coruros inhabit extensive systems of self-constructed tunnels and harvesting of geophytes through tunnelling appears to be their primary mode of foraging, albeit there is also evidence of foraging on vegetation around the tunnel opening (Reig 1970, Contreas et al. 1993, Torres-Murra & Contreas 1998, Begall & Gallardo 2000, Urejolla et al. 2005). Thus, the coruros spend most of their time in lightless underground ecotope and occasional above-ground forays likely provide less occasions to "fear" a fall.

The nesting experiments provided no evidence for visually guided spatial orientation. As noted above (see Methods), a robust spontaneous motivation to build nest in a particular sector of a circular arena has been reported in several species of bathyergid mole-rats (Burda et al., 1990a,b Oliveriusová et al. 2012). In the absence of salient visual, tactile and olfactory cues, mole-rats use magnetic field azimuth as the primary orientation cue. This nesting assay has been successfully applied to study the physiological mechanisms of magnetic compass orientation (Marhold et al. 1997, Němec et al. 2001, Thalau et al. 2006, Wegner et al. 2006, Burger et at. 2010). Here, we modified this assay by placing a prominent, familiar visual cue – white cue card between outer and inner walls of the arena and found that azimuthal position of the cue card has no effect on directional preference in the giant mole-rat. Since rodents such as rat and mouse tend to establish nests/home bases near salient visual cues (Clark et al. 2005, 2006, Hines & Whishaw 2005), it is tempting to conclude that the giant mole-rat either do not see the cue or, more likely, do not use visual cues for spatial orientation. But this inference is premature. The assay is entirely based on the spontaneous motivation to use a particular cue for orientation in an arena. Although we tried to associate the cue card with a nest position in the pre-training period (see Methods), it remains unclear whether mole-rats would spontaneously use the cue card for orientation if they were capable to see it. Actually, a random orientation of mole-rats in our experiments might, hypothetically, be consequence of conflict between visual and magnetic cues. Hence, the negative results of the performed behavioural tests are inconclusive. Experiments, in which no appetitive motivation is required, will be needed to assess the capacity for visually guided spatial orientation in the bathyergid mole-rats. As the bathyergid mole-rats are known to be able to swim (Hickman 1983, 1988), the Morris water-maze (Morris 1984) seems to be particularly promising experimental paradigm for future research.

Conclusion

Behavioural tests performed in this study revealed starkly different visual capabilities in the fossorial coruro and the strictly subterranean African mole-rats. While the coruro seems to possess visual capacities comparable with that of surface-dwelling rodents, vision is severely impaired in the bathyergid mole-rats. In line with previous anatomical and behavioural evidence (see Introduction), these findings support the hypothesis that low acuity residual vision play an indispensable role in bathyergid anti-predatory behaviour and tunnel maintenance but not in spatial orientation.

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Balikpapan, Indonesia, September – October 2008, field research on ecology of proboscis monkey (*Nasalis larvatu*s) in Eastern Kalimantan

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AWARDS:

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TEACHING:

During 2008-2012 I participated in teaching of following courses at University of South Bohemia: **Summer School of Behavioural Methods, Field work Practices (Zoology), Zoology of Vertebrates Practices**.

I was main organiser of Field Vertebratology Excursion for 50 students: **Greece - Thrakia 2011**

Student supervision: I was a supervisor of a bachelor thesis of a student from University of South Bohemia (Fremlová 2013) and reviewed undergraduate thesis of students from Charles University (Krejčová 2010) and Masaryk University (Fialová 2014, 2015).

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