



Light propagation in burrows of subterranean rodents

RNDr. Thesis

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Anotation: This RNDr. thesis focuses on light propagation in burrows of subterranean rodents. 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 anelli* 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.

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Ondřej Kott

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; Nĕmec *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. Nĕmec *et al.*, 2007, 2008; Kott, Šumbera & Nĕmec, 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 cm², 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

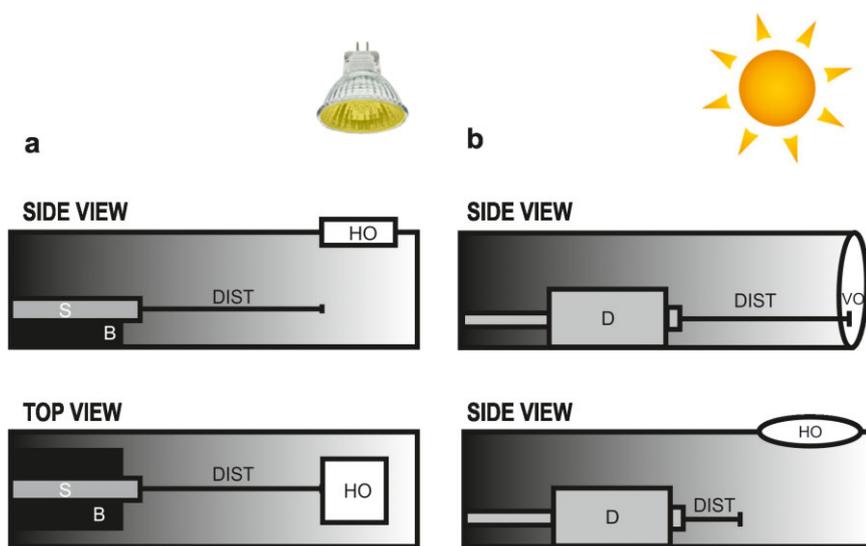


Figure 1 The paradigms used in this study. (a) An artificial burrow used for light level measurements. (b) Measurements of light propagation in Ansell's mole-rat (*Fukomys ansellii*) burrows. B, a wooden block serving as a stand for the spectrometer; D, datalogger with embedded quantum sensors; DIST, 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 $\mu mol photons s^{-1} m^{-2}$. The collection area of the sensor was 0.25 cm², 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} ($\sim 4.7 \times 10^{11}$ 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 $\sim 3.77 \times 10^{-4} Rh^* rod^{-1} s^{-1}$ (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; Nĕmec, 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 $\sim 30 Rh^* rod^{-1} s^{-1}$ (Deans *et al.*, 2002). In the rat, rod saturation occurs at $\sim 4 \times 10^3 Rh^* rod^{-1} s^{-1}$ (Nakatani, Tamura & Yau, 1991). In a transgenic mouse whose retina lacks cones, the ganglion cell response attenuates greatly at the intensity of $\sim 10^4 Rh^* rod^{-1} s^{-1}$ 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):

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

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.

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

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: $S_{retina} = 3.19 mm^2$, $S_{pupil} = 0.108 mm^2$ and $0.008 mm^2$ for a dilated and constricted pupil, respectively, $d = 3.25 \mu m$, $L = 10 \mu m$ (Peichl *et al.*, 2004; Nĕmec, 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

Spectral range (nm)	Distance from burrow opening (cm)							
	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 $\text{s}^{-1} \text{cm}^{-2}$).

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 $\text{s}^{-1} \text{cm}^{-2}$ 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 $\text{s}^{-1} \text{cm}^{-2}$ 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 $\text{s}^{-1} \text{cm}^{-2}$ during a sunny day and between $\sim 2 \times 10^{13}$ and $\sim 6 \times 10^{12}$ photons $\text{s}^{-1} \text{cm}^{-2}$ during a moonlit night (Fig. 3a). Blue light irradiance ranged between $\sim 2 \times 10^{13}$ and $\sim 6 \times 10^{12}$ photons $\text{s}^{-1} \text{cm}^{-2}$ during the day and was $\leq 6 \times 10^{12}$ photons $\text{s}^{-1} \text{cm}^{-2}$ 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 $\text{s}^{-1} \text{cm}^{-2}$ during the day and was $< 10^{13}$ photons $\text{s}^{-1} \text{cm}^{-2}$ 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 $\text{s}^{-1} \text{cm}^{-2}$, respectively. The photon irradiance needed for rod saturation is $\sim 2.28 \times 10^{14}$ photons $\text{s}^{-1} \text{cm}^{-2}$, provided that rod saturation occurs at $10^4 \text{ Rh}^* \text{ rod}^{-1} \text{ s}^{-1}$. In other words, light levels $> 2.28 \times 10^{14}$ photons $\text{s}^{-1} \text{cm}^{-2}$ constitute the photopic range, light levels $< 5 \times 10^{10}$ photons $\text{s}^{-1} \text{cm}^{-2}$ the scotopic range and light levels between 5×10^{10} and 2.28×10^{14} photons $\text{s}^{-1} \text{cm}^{-2}$ 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

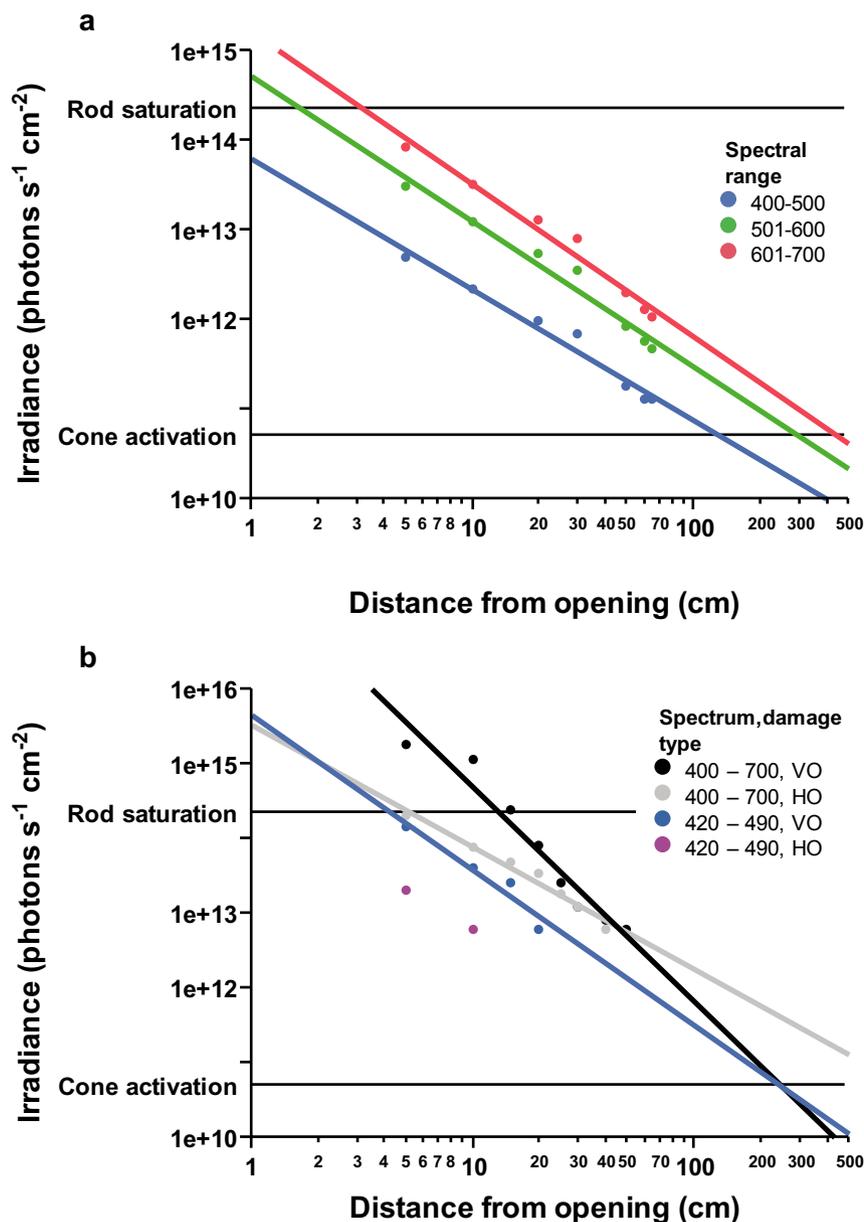


Figure 2 Light propagation within the artificial burrow (a) and the natural burrows of Ansell's mole-rat (b). The two horizontal lines mark light levels needed for cone activation and rod saturation, the zone between them represents mesopic light levels, at which both cones and rods contribute to vision. 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), damage type	Distance from burrow opening (cm)							
	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.

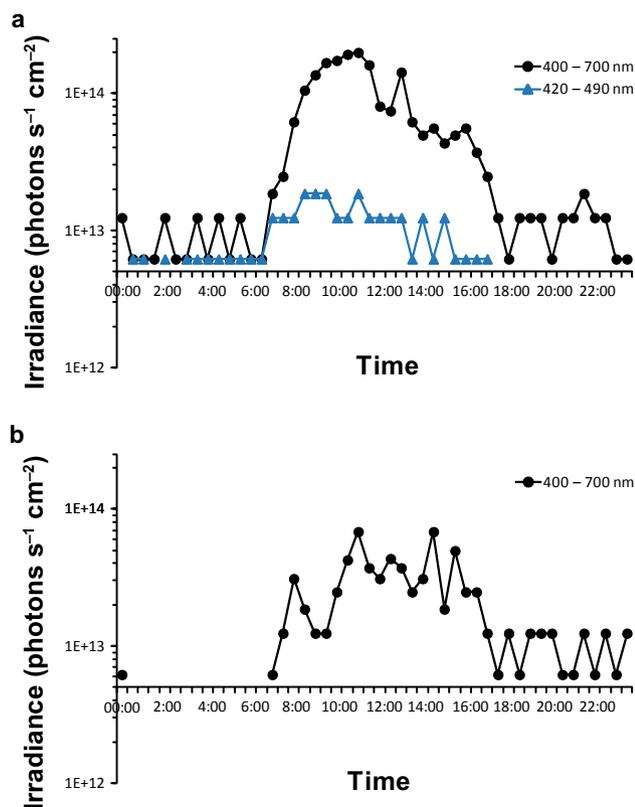


Figure 3 Light level measured over a 24 h period. Measurements were taken 15 cm from the opening in the vertically (a) and horizontally (b) breached burrows. The missing data points represent intensities that were below the threshold of the datalogger used for the field measurements.

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	$I_{rr} = 6.270 \times 10^{13} \times \text{Dist}^{-1.436}$	0 cm	132 cm	2961 m
501–600	$I_{rr} = 5.244 \times 10^{14} \times \text{Dist}^{-1.624}$	2 cm	229 cm	3118 m
601–700	$I_{rr} = 1.637 \times 10^{15} \times \text{Dist}^{-1.703}$	3 cm*	448 cm*	3374 m*
Foraging burrow				
400–700, VO	$I_{rr} = 3.591 \times 10^{17} \times \text{Dist}^{-2.869}$	13 cm*	245 cm*	125 m*
400–700, HO	$I_{rr} = 3.306 \times 10^{15} \times \text{Dist}^{-1.634}$	5 cm*	891 cm*	8886 m*
420–490, VO	$I_{rr} = 4.496 \times 10^{15} \times \text{Dist}^{-2.077}$	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; I_{rr}, 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.