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Photosensitivity to different light intensities in blind and sighted rodents

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SUMMARY

Photoperiod is an important cue regulating biological rhythms in mammals, including 'blind' subterranean and sighted fossorial rodent species. These species may respond differentially to changes in light quality according to their retinal complexity. The effects of increasing light intensity on daily rhythms of urine excretion and urinary output of 6-sulfatoxymelatonin levels were compared in 'blind' mole rats *Spalax ehrenbergi* and sighted social voles, *Microtus socialis*. Our results show that the threshold irradiance required to entrain rhythms of voles is three magnitudes greater than that for mole rats. The results suggest that mole rats have an operational photoreceptive pathway with a lower threshold irradiance than voles. Such a low threshold reflects the remarkable capability of this 'blind' species to utilize light signals even under challenging light conditions.

Key words: cosinor analysis, daily variation, melanopsin, photophase irradiance, retinal photoreceptors, ultradian rhythm, urinary melatonin.

INTRODUCTION

Rhythmicity in biological functions has been described in several rodent species, including social voles, *Microtus socialis* (Haim et al., 2005; Zubidat et al., 2007) and the 'blind' mole rats, *Spalax ehrenbergi* (Ben-Shlomo et al., 1996b; Tobler et al., 1998). The cycles of day and night and the annual pattern of changing day lengths generate a reliable photosignal for temporal coordination of a wide array of circadian and seasonal functions, respectively, in mammals living outside the tropics (Goldman, 2001). In photoperiodic species, light signals are perceived by photoreceptors localized in the retina and conveyed to the hypothalamus by the retinohypothalamic tract; this information is then conveyed to the pineal gland. Eventually, this photosignal transduction cascade encodes photoperiodic information in rhythmic secretions of the pineal hormone melatonin (MEL). MEL synthesis and secretion are mainly restricted to the dark period; both the amplitude and duration of secretion reflect seasonal changes in night length. Consequently, the nightly MEL rhythm provides a neurohormonal clock which allows individuals to correctly track day length and to time their internal environment in anticipation of impending changes associated with the time of day and the season of the year (Pévet, 2003; Hazlerigg and Wagner, 2006).

The impact of the quality of lighting (irradiance and wavelength) on physiology and behavior has been demonstrated for various species (Nelson and Takahashi, 1991; Griffith and Minton, 1992; Aral et al., 2006). Overall, individuals of each species appear to respond differently to changes in light quality and have distinct spectral and irradiance thresholds to optimize survival. The mammalian retina contains two distinct types of photoreceptors; visual photoreceptors (VPRs) and non-visual photoreceptors (NVPRs) (Kavakl and Sancar, 2002). Studies in humans and rodents with VPR deficiency have suggested that the classic VPRs are obligatory for precise vision and image perception, but are not mandatory to synchronize the circadian clock. Conversely, the NVPRs play an important role in synchronizing circadian rhythms to the light–dark cycle (Freedman et al., 1999; Klerman et al., 2002).

The solitary 'blind' mole rat, *S. ehrenbergi*, is strictly a fossorial species that exhibits an extreme adaptation to subterranean existence (Nevo, 1988). Social voles are semi-fossorial rodents spread throughout the Mediterranean grasslands and cultivated fields in Israel (Harrison and Bates, 1991). Both *M. socialis* and *S. ehrenbergi* use ambient photic information for entrainment of several biological rhythms (Haim et al., 1983; Rado et al., 1992; Goldman et al., 1997; Haim et al., 2005; Zubidat et al., 2007). Whilst the retina of social voles has normal neural projections to the circadian clock (both VPR and NVPR pathways), the eyes of mole rats are severely degenerated and the vestigial retina only displays NVPR projections.

These profound differences in retinal photoreceptors and ambient conditions between the two species have led to our hypothesis that if NVPRs are strongly related to circadian photoreception, then *S. ehrenbergi* exposed to extremely low irradiance will respond with much higher photosensitivity than *M. socialis*. The two study species were chosen for comparison because their biological rhythms and melatonin parameters are well characterized in the laboratory (Zubidat et al., 2007; Ben-Shlomo et al., 1996a). Even though *M. socialis* and *S. ehrenbergi*, which belong to the *Myomorpha* suborder, are not closely related, differences in their retinal anatomy and illumination habitat offer suitable and interesting models for evaluating the impact of increasing photophase intensity on retinal light-entrainable rhythms. To compare the sensitivity of the photoreception pathways of social voles and 'blind' mole rats we measured daily responses in urine production rate and urinary MEL concentration, as markers for biological rhythmicity, to increasing light intensity of the same irradiation wavelength. Daily urine production rate has been adopted as an index for rhythmicity because it is well established that its rhythm exhibits clear photoperiod-mediated circadian oscillation in humans and rodent species, including social voles, with generally high levels during the active period and low levels during the inactive period (Mills, 1951; Ratte et al., 1974; Zisapel et al., 1999; Schibler et al., 2003; Chen et al., 2004; Zubidat and Haim, 2007). Additionally, the procedure is non-

invasive and easily conducted, making it particularly appealing for the investigation of daily rhythmicity in small rodent species.

MATERIALS AND METHODS

Animals

Male social voles *M. socialis* Pallas 1773 (62±1.2 g; 3–4 months of age) were obtained from our breeding colony maintained at the animal facility in Oranim, University of Haifa, Israel. The males were the second and third generation of voles born to a colony established from wild mating pairs that were randomly collected during the autumn plowing season from cultivated alfalfa fields in the Beit Sheaan Valley. Male 'blind' mole rats *S. ehrenbergi* Nehring 1898 (256±8.5 g) of 2n=60 chromosomal population (Nevo et al., 2001), were caught in agricultural fields located in the area around Rehovot (31°53′33.98″N, 34°48′40.58″E; Central District of Israel) during the winter and spring. Before experiments began and when animals were not being tested, individuals from both species were kept under controlled ambient conditions of 25±2°C and equatorial photoperiod (12 h L:12 h D) with 125 μW cm^{-2} of broadband fluorescent lights (40 W) and relative humidity (RH%) of 60%. Before and during experiments animals were housed individually in transparent polycarbonate cages (430 mm×230 mm×260 mm) filled with approximately 200 mm of sawdust provided as bedding. Rodent food pellets (Koffolk, Tel Aviv, Israel; 21% crude protein, 4% crude fat, 4% cellulose, 13% moisture, 7% ash, 18.7 kJ g^{-1} gross energy) and carrots were provided *ad libitum*. Additionally, mole rats were provided with apples and sweet potato tubers. All supplemental feeding was randomly timed in order to prevent diet-entrainable responses that could compromise the results of the experiments. All animals were housed and tested according to institutional regulations for experimental animals and all studies were approved by the Ethics and Animal Care Committee at the University of Haifa.

The experiment was designed to quantify species responses to different exposures to yellow light (586 nm) of increasing intensity during the photophase. To this end, an incandescent lamp (*N*=8 lamps in the controlled room; 40 W, OSRAM, Molsheim, France) was adjusted to about 300 mm above each cage floor in an environmentally controlled room. All lamps were connected to a dimmer circuit (230 V AC; Fetaya Ltd, Rishon Le Zion, Israel) and were manually adjusted to the desired light intensity. Intensity level was measured by a hand-held fiber optic spectrometer (AvaSpec-2048-FT-SDU, Avantes, Eerbeek, The Netherlands) while placing the light sensor directly beneath the lamp at an equal distance between the lamp and the cage floor. The light intensity of each lamp was read at 10 s intervals for 60 s and values of all lamps were averaged to estimate the mean light intensity inside the climate-controlled environmental room. Five different mean light intensities were used in the experiments presented here: 73±7, 147±10, 293±11, 366±11 and 498±15 μW cm^{-2}.

Urine collection

Urine samples of all individuals of each species were collected at 4 h intervals for a 24 h period. To this end, animals at the end of 3 weeks of acclimation were transferred to special cages (480 mm×375 mm×210 mm) equipped with a wire mesh (7 mm×7 mm spacing) bottom platform (TECNIPLAST S.p.a, Buguggiate, Italy). Urine collection cages were placed in the same environmental room in which animals were exposed to the experimental conditions. Each cage was positioned ~20 mm above a plastic plate using a special metallic rack. The gathered urine spots on the plate were transferred to Eppendorf tubes by Pasteur pipettes. The mass of each urine sample was obtained directly, at the end of urine collection, using a milligram Satorius balance (ED623S, Goettingen, Germany; ±0.001 g). Urine volume was calculated by dividing sample mass by urine specific gravity; although this parameter was not measured, it was assumed to be 1 g ml^{-1} as documented previously (Schoorlemmer et al., 2001; Tendron-Franzin et al., 2004). Urine samples were frozen at –20°C for later analysis.

Urinary MEL assay

Urine samples were analyzed for MEL by measuring its main urinary metabolite (6-sulfatoxymelatonin) levels (Stieglitz et al., 1995). Quantitative determination of 6-sulfatoxymelatonin was conducted by a solid phase enzyme-linked immunosorbent assay (ELISA; IBL, Hamburg, Germany; cat. no. RE54031). The intra-assay and inter-assay coefficients of variation were 5.8–204 ng ml^{-1} (5.2–12.2%) and 12.4–220 ng ml^{-1} (5.1–14.9%), respectively. Samples (10 μl aliquots) were subjected to the ELISA method with duplicate determinations as described previously (Zubidat and Haim, 2007). Thereafter, 6-sulfatoxymelatonin concentrations (ng ml^{-1}) were spectrophotometrically measured at a wavelength of 450 nm by an automated ELISA system including microplate absorbance reader (SunRise; Tecan, Grödig, Austria) and data were analyzed by Magellan™ data analysis software (Tecan).

Statistical analysis

Data were analyzed for statistical significance by ANOVA models at the $P<0.05$ level using SPSS 13.0 for windows (SPSS Inc., Chicago, IL, USA). All values are expressed as means ± s.e.m. Data of daily variation in urine production and urinary MEL levels were subjected to two-way factorial mixed-model ANOVA (MANOVA). The MANOVA model tested for mean effects of irradiance (five levels) between-subjects and time (seven levels) within-subjects, and time×irradiance interaction effects. Repeated measures one-way ANOVA (RMANOVA) was also completed for the data within each irradiance group if relevant effects of either time or interactions in the MANOVA design were found to be statistically significant. Bonferroni correction *post hoc* pairwise comparisons were applied to the data following significant RMANOVA. One-way ANOVA followed by Tukey *post hoc* test was also used to test for significant differences between total daily urine production and mean daily urinary MEL levels collected under the different intensities. One-tailed paired Student's *t*-tests were used to make statistical comparisons between day and night levels for each irradiance group. To this end, the *P*-value threshold was adjusted for multiple comparisons ($P<0.05/5$). The relationship between increasing irradiance and each of total daily urine production and mean daily urinary MEL concentration of both species was computed by the Pearson correlation coefficient (*R*).

Daily urine production and urinary MEL levels of each individual under all irradiance exposures were also fitted for rhythmicity by the cosinor method (Nelson et al., 1979; Minors and Waterhouse, 1989). This method is a non-linear curve fitting regression that computes and minimizes point by point sum of squared residuals for a set of observed data. The best achieved minimization of least squares represents the best cosine fitted equation of approximating a curve to the entire raw data over a trial period.

The cosine curve best approximating the data over a given period is described by the following equation:

$$F(t) = \text{Mesor} + \text{Amplitude} \times \cos\left[2\pi \times \frac{(t - \text{Acrophase})}{\text{Period}}\right], \quad (1)$$

where $F(t)$ is the urine production rate or urinary MEL concentration at time t of the best fitted cosine equation defined by mesor (the rhythm-adjusted mean based on the parameters of the best least squares curve fitting), amplitude (half the difference between the crest and trough of the best least squares curve fitting), acrophase (the crest time with reference to local midnight, 00:00 h, of the best least squares curve fitting) and period (length of a complete cycle). A significant rhythm is detected when the null hypothesis that the variances of the cosine best fitted model and those of the linear model are equal (amplitude equal zero) is rejected by an F-test statistic at the $P<0.05$ level. The Bingham test was used for statistical comparison between group mean rhythm parameters, of both variables, estimated under each experimental condition (Bingham et al., 1982). Cosinor analysis was performed using TSA-Time Series Analysis Serial Cosinor 6.3 software package (Expert Soft Technology, Esvres, France).

RESULTS

Urine daily rhythms

In *M. socialis*, clear time-dependent variations ($F_{6,168}=29.62$, $P<0.0001$), time×irradiance interactions ($F_{24,168}=2.9$, $P<0.0001$) and irradiance effects ($F_{4,28}=17.34$, $P<0.0001$) were indicated by the MANOVA. Conversely, split analyses of the effect of time on urine production rates under each irradiance showed no significant effects under either 73 or 147 μW cm^{-2}; however, robust time effects were established under higher intensities (293, 366 and 498 μW cm^{-2}; Fig. 1). Moreover, total daily urine production rates showed significant light intensity variations (one-way ANOVA: $F_{4,28}=13.38$, $P<0.0001$)

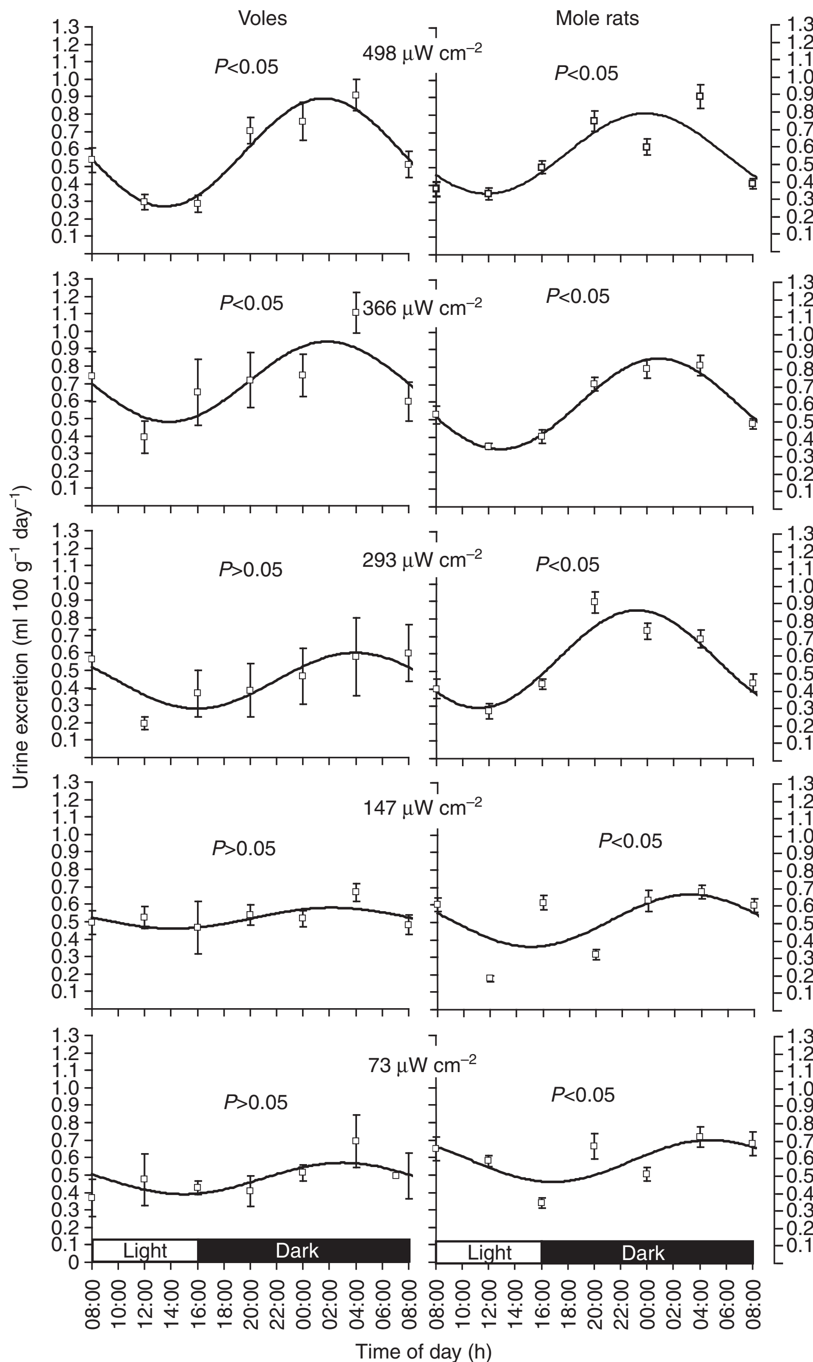

Fig. 1. Effect of different light intensities on daily rhythms in urine production rates of social voles and 'blind' mole rats. Values are means ± s.e.m. of N=8 voles and N=6 mole rats. Solid lines represent the best fitting cosine curve to the entire data of each experimental group over a 24 h period. White and black bars represent the alternation between photophase and scotophase periods, respectively. $P<0.05$ indicates that the amplitude of the rhythm significantly differs from zero in value.

Table 1. Analyses of urine excretion rates of voles and mole rats under varying light intensity

	Irradiance ($\mu W\,cm^{-2}$)	One-way ANOVA	Mesor ($ml\,100g^{-1}\,h^{-1}$)	Amplitude ($ml\,100g^{-1}\,h^{-1}$)	Acrophase (h:min)	PR (%)	
M. socialis		$F_{6,42}$; *P*					$F_{2,6}$; *P**
	73	0.87; 0.52	0.48^{a,b} [0.40–0.56]	0.09^{a} [0.03–0.22]	02:58 [00:31–06:26]	3.78	3.32; 0.36
	147	0.9; 0.51	0.52^{a,b} [0.47–0.59]	0.06^{a} [0.03–0.15]	02:14 [01:05–05:31]	3.09	0.74; 0.44
	293	1.12; 0.37	0.44^{a} [0.32–0.56]	0.16^{a,b} [0.02–0.33]	03:56 [00:31–08:24]	6.06	1.5; 0.19
	366	2.99; 0.02	0.71^{b} [0.60–0.82]	0.23^{b,c} [0.07–0.39]	01:51 [00:49–04:31]	13.79	11.34; 0.02
	498	9.78; 0.0001	0.58^{a,b} [0.52–0.64]	0.32^{b,c} [0.23–0.40]	01:32 [00:34–02:30]	49.24	19.25; 0.0001
S. ehrenbergi		$F_{6,30}$; *P*					$F_{2,4}$; *P**
	73	10.44; 0.0001	0.58 [0.53–0.63]	0.12^{a} [0.05–0.19]	04:46^{a} [02:17–07:16]	22.02	23.92; 0.008
	147	39.5; 0.0001	0.51 [0.45–0.56]	0.15^{a} [0.07–0.24]	03:09^{a} [01:10–05:07]	29.91	24.79; 0.002
	293	66.79; 0.0001	0.57 [0.53–0.62]	0.28^{b} [0.22–0.34]	23:12^{b} [22:20–00:04]	68.14	68.11; 0.0001
	366	50.56; 0.0001	0.59 [0.56–0.62]	0.26^{b} [0.21–0.31]	00:50^{c} [00:11–01:31]	74.02	95.44; 0.0001
	498	54.98; 0.0001	0.58 [0.53–0.63]	0.23^{b} [0.16–0.31]	23:52^{b,c} [22:40–01:04]	49.9	63.43; 0.0001

Values in brackets for mesor, amplitude and acrophase are 95% confidence intervals of the group mean.

PR (percentage of the rhythm) represents the proportion of the total variance of the data accounted for by the cosine approximation.

**P*-value for rejection of the zero amplitude hypothesis at $P<0.05$. Different letters represent significant differences between treatments for each species ($P<0.05$).

and Tukey *post hoc* analysis indicated that mean total values under 293 $\mu W\,cm^{-2}$ (17.10±0.91 $ml\,100\,g^{-1}\,h^{-1}$) were significantly ($P<0.05$) higher than those under other intensities.

The cosinor analysis results are presented in Table 1 and they are consistent with the ANOVA analyses, in which clear 24 h rhythms were established at 293, 366 and 498 $\mu W\,cm^{-2}$, but not under the lower intensities of 73 and 147 $\mu W\,cm^{-2}$. Significant increases in mesor levels were estimated at 366 $\mu W\,cm^{-2}$ in comparison with the other light intensities. Increased light intensity resulted in wider amplitudes, but these differences did not reach the fixed significance level. All acrophase occurrences were in the first hours of scotophase at about 18:00 h, except at 293 $\mu W\,cm^{-2}$ when the acrophase was delayed by ~2 h (Table 1).

In *S. ehrenbergi*, MANOVA also revealed significant effects of time ($F_{6,150}$=104.47, $P<0.0001$) and time×irradiance interactions ($F_{24,150}$=21.35, $P<0.0001$) on urine production rates, but no significant effect of irradiance was obtained ($F_{1,25}$=0.67, P=0.62). Consistent with these data, night-time urine production rates were consistently higher than daytime rates under each irradiance tested, but no significant differences were detected in total daily urine levels among the five light intensity groups (one-way ANOVA; $F_{4,25}$=1.04, P=0.41).

Significant time-dependent variation in urine production rates for all irradiance groups was also detected by the cosinor procedure. However, neither mesor nor amplitude was affected by the increasing light intensity, whereas the acrophase was significantly advanced by ~4 h in response to increasing intensity above 147 $\mu W\,cm^{-2}$ (Table 1).

MEL daily rhythms

MANOVA showed that time ($F_{6,180}$=25.98), irradiance ($F_{4,30}$=31.34) and the time×irradiance interaction ($F_{24,180}$=7.46) significantly ($P<0.0001$) affected urinary MEL daily rhythms of *M. socialis*. The 24 h MEL profiles showed significant (one-way ANOVA; Table 2) time-related variation under all light intensities with higher levels during the scotophase than during the photophase (Fig. 2). Generally, daily MEL levels increased as irradiance increased from 73 $\mu W\,cm^{-2}$ to 498 $\mu W\,cm^{-2}$, and levels (0.81±0.1 $ng\,ml^{-1}$) under the lowest intensity significantly ($P<0.001$) differ from those (2.37±0.09 $ng\,ml^{-1}$) under the highest intensity. No statistically significant differences were observed between the other irradiance levels. Also, greater day–night differences were associated with increased irradiance levels during the photophase, and these differences under the highest irradiance were about 80% greater than those under the lowest irradiance.

Similarly, in *M. socialis*, significant 24 h rhythms were detected by the cosinor analysis in MEL levels under all irradiance treatments, except under 73 $\mu W\,cm^{-2}$, and the highest percentage of the rhythm (PR) was estimated under 293 $\mu W\,cm^{-2}$ (55.84%; Table 2). In general, both mesor and amplitude levels increased as irradiance increased, except for voles exposed to 366 $\mu W\,cm^{-2}$. Under all irradiance treatments, the acrophase occurrence was recorded shortly before lights off (08:00 h), except under the highest irradiance level in which the acrophase was significantly advanced by about 4–6 h (00:53 h).

Likewise, the 24 h MEL profile for *S. ehrenbergi* showed significant ($P<0.0001$) time ($F_{6,150}$=6.54), irradiance ($F_{4,25}$=118.08) and time×irradiance interaction ($F_{24,150}$=2.85) variations when subjected to MANOVA analysis. However, one-way ANOVA and cosinor split analyses detected significant 24 h rhythms only for animals exposed to 293 $\mu W\,cm^{-2}$ during the photophase (Table 2). The highest PR of the 24 h rhythm (35.28%) was estimated under this irradiance; whereas those under the remaining irradiance levels did not exceed 6%. Interestingly, in animals exposed to 73 $\mu W\,cm^{-2}$ during the photophase a robust ultradian rhythm of 12 h (~45% PR) was detected by the cosinor analysis, but no other ultradian rhythms were detected for either species under the different irradiance conditions.

A significant decrease in MEL levels was observed in relation to increased irradiance; under the lowest irradiance, mean daily MEL levels were 3.56±0.13 $ng\,ml^{-1}$ compared with 0.41±0.07 $ng\,ml^{-1}$ under the peak irradiance levels. Likewise, day–night differences in MEL levels of mole rats were less pronounced as irradiance increased (Fig. 2). The cosinor analysis showed that both mesor and amplitude levels of the 24 h rhythms were affected by the increasing intensity, as both estimates were significantly decreased as irradiance increased (Table 2). The first acrophase of the 12 h ultradian rhythms for animals exposed to 73 $\mu W\,cm^{-2}$ occurred at 20:43 h.

There were no significant correlations between increasing light intensity and total daily urine production rates of either species (Fig. 3; Pearson correlation: R=0.25, $P>0.05$, N=40, and R=0.02, $P>0.05$, N=30, for voles and mole rats, respectively). In *M. socialis*, mean daily urinary MEL concentration significantly and positively varied with increasing intensity (R=0.84, $P<0.05$,

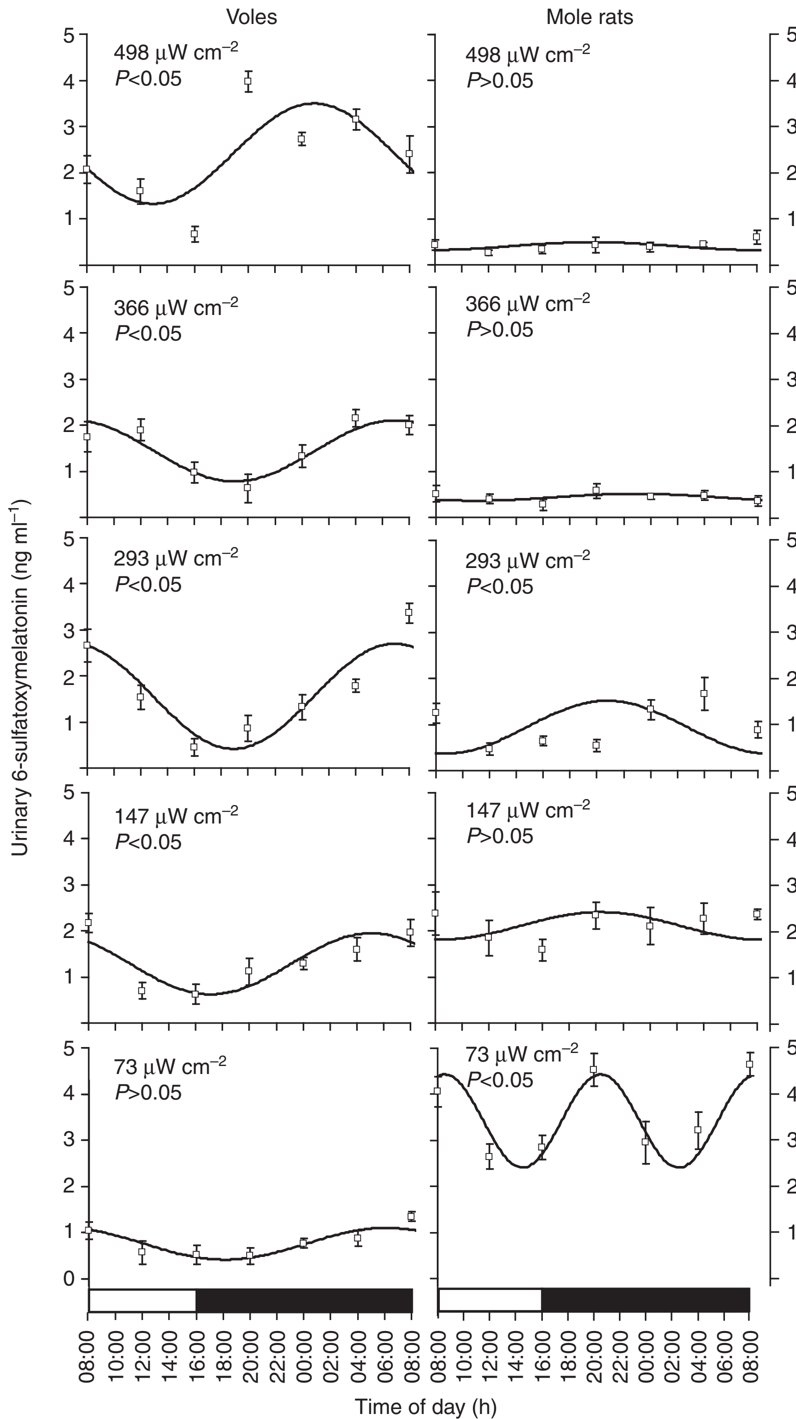

Fig. 2. Urinary melatonin daily rhythms of social voles and 'blind' mole rats exposed to increasing light intensity during the photophase. Values are means ± s.e.m. of N=7 voles and N=6 mole rats. Solid lines represent the best fitting cosine curve to the entire data of each experimental group. All rhythms are of 24 h period, except for mole rats exposed to 73 μW cm^{-2}, for which the period is 12 h.

N=35), whereas in *S. ehrenbergi* a significant, negative correlation was found between the two parameters (R=0.90, P<0.05, N=30; Fig. 3).

DISCUSSION

Blind mole rats, *S. ehrenbergi*, represent an extremely solitary and seasonally breeding species that displays many adaptations to the challenging subterranean environment (Nevo, 1991; Nevo, 1995). One of the most remarkable adaptations is the ability of this species to discriminate between light and dark ambient lighting and to entrain the internal circadian clock accordingly (Negroni et al., 1997; Tobler et al., 1998). Clearly, the photoperiodic responses of *S. ehrenbergi* under the exceptional light characteristics of the subterranean environment are of interest.

The most interesting finding of this study is the extraordinary ability of the 'blind' mole rats to use as low as 73 μW cm^{-2} of incandescent light to adjust daily variations in urine production and MEL levels to the light–dark cycle. Mole rats exposed to the lowest irradiance level during the photophase exhibited clear ultradian (12 h) and circadian rhythms in MEL and urine production levels, respectively. Increasing intensities, however, significantly decreased mean urinary MEL levels, but had similar effects on total daily urine volume. In contrast to these results, light intensities lower than 293 μW cm^{-2} were not sufficient for

Table 2. Analyses of urinary MEL levels of voles and mole rats under varying light intensity

	Irradiance (μW cm^{-2})	One-way ANOVA	Mesor (ng^{-1} ml^{-1})	Amplitude (ng^{-1} ml^{-1})	Acrophase (h:min)	PR (%)	
M. socialis		$F_{6,36}$; P					$F_{2,6}$; P*
	73	4.42; 0.002	0.76^{a} [0.62–0.90]	0.34^{a} [0.15–0.53]	06:08^{a} [03:46–08:28]	22.35	9.05; 0.30
	147	8.09; 0.001	1.29^{b} [1.09–1.48]	0.66^{a,b} [0.39–0.93]	05:07^{a} [03:29–06:43]	43.47	23.16; 0.001
	293	16.23; 0.0001	1.56^{b} [1.33–1.78]	1.14^{c} [0.84–1.44]	06:56^{a} [05:47–08:04]	55.84	92.14; 0.0001
	366	4.87; 0.001	1.45^{b} [1.25–1.64]	0.66^{a,b} [0.29–0.92]	07:00^{a} [05:17–08:40]	34.04	21.94; 0.001
	498	16.91; 0.0001	2.42^{c} [2.13–2.70]	1.09^{b,c} [0.67–1.50]	00:53^{b} [00:29–02:16]	37.37	19.15; 0.001
S. ehrenbergi		$F_{6,30}$; P					$F_{2,4}$; P*
	73	5.57; 0.001	3.42^{a} [3.15–3.69]	1.01 [0.65–1.37]	20:43† [17:56–23:29]	45.04	27.43; 0.005
	147	0.94; 0.48	2.11^{b} [1.85–2.37]	0.30^{a} [–0.09–0.69]	21:41 [17:24–23:02]	6.00	2.69; 0.3
	293	5.04; 0.001	0.94^{c} [0.77–1.11]	0.57^{a} [0.32–0.83]	21:15 [20:51–23:39]	35.28	17.48; 0.001
	366	0.95; 0.49	0.44^{c} [0.34–0.54]	0.08^{b} [–0.07–0.22]	23:04 [20:15–22:01]	2.73	1.22; 0.58
	498	1.17; 0.35	0.41^{c} [0.32–0.50]	0.09^{b} [–0.05–0.22]	20:19 [17:36–00:46]	4.31	1.99; 0.43

Values in brackets for mesor, amplitude and acrophase are 95% confidence intervals of the group mean.

PR (percentage of the rhythm) represents the proportion of the total variance of the data accounted for by the cosine approximation.

**P*-value for rejection of the zero amplitude hypothesis at $P<0.05$. Different letters represent significant differences between treatments for each species ($P<0.05$).

†First acrophase of the 12 h ultradian rhythm.

entraining urine production rates of the fully sighted fossorial *M. socialis* and generally the excreted levels were much higher in voles exposed to light intensities greater than 293 μW cm^{-2}. Moreover, our results show that voles exposed to higher intensities had greater urinary MEL concentrations than voles exposed to lower intensities.

In addition to the fundamental circadian variation in the frequency of pineal MEL secretion, nocturnal ultradian or episodic secretory activities of the gland have also been described in human and non-human species, including rodents (Pang and Yip, 1988; Chan et al., 1991; Geoffriau et al., 1999; Salti et al., 2000). These ultradian rhythms in nocturnal MEL have been suggested to be associated with REM sleep stage in humans, but the significance of these ultradian MEL rhythms in rodent species remains undetermined. The observed 12 h ultradian rhythm in the 'blind' mole rats is characterized by acrophase occurring around the onset and the offset of the scotophase. An ultradian rhythm of MEL secretion with two peaks, one in the evening and the other in the morning, has also been reported previously (Arendt, 1985; Wehr et al., 1995; Nakahara et al., 2003). The bimodal rhythm of MEL has been suggested to reflect the separate regulation of two different oscillators. One limitation of this finding in our study is the relatively large sampling time bins (4 h intervals) and thus higher specimen sampling frequencies are essentially required for more reliable ultradian spectral analysis. The observed dual pattern in MEL secretion, however, focuses on the complexity of the pineal secretory activity, which most likely is influenced by the features of light during the photophase.

It remains unspecified how the individuals of the 'blind' species perceive the light signal, but recent studies suggest an important photoreception role for a novel photopigment, melanopsin, in its vestigial retina (Hannibal et al., 2002). It is well established that all melanopsin-containing retinal cells are highly sensitive to light (Berson et al., 2002). Therefore, the photoperiod-related variations observed here in both MEL and urine excretion, as well as in other parameters (Haim et al., 1983; Rado et al., 1992; Ben-Shlomo et al., 1996b; Berson et al., 2002), are likely to be regulated by the melanopsin photoreceptors.

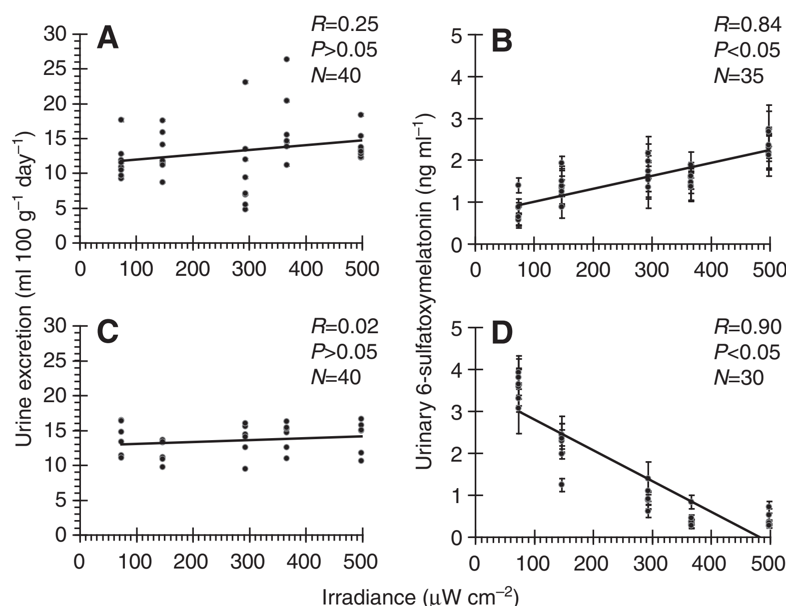

Fig. 3. Relationship between irradiance and urine excretion and urinary melatonin levels of social voles (A,B) and 'blind' mole rats (C,D). Results are total daily urine production and means ± s.e.m. of daily urinary MEL concentrations of individuals from each species. Pearson coefficient (*R*), *P*-value and *N* of the correlation test are presented for each experimental group.

Our results demonstrate that irradiance levels during the photophase significantly affected the scotophase MEL responses of both *M. socialis* and *S. ehrenbergi*. Interestingly, light of increasing intensity evoked opposite effects on urinary MEL levels of the two species (Fig. 3). In *M. socialis*, mesor and amplitude levels were increased and rhythms were statistically significant, whereas in *S. ehrenbergi*, mesor and amplitude levels were lessened and rhythms were unrecognizable. Moreover, the results of the present study also demonstrate that the maximum effective light intensity for modifying urinary MEL rhythms in both species was 293 μW cm^{-2}, as the highest percentage of urinary MEL rhythms of both voles and mole rats was observed at this light level. However, our data indicate that the threshold irradiance for controlling MEL and urine levels in the mole rats is lower (73 μW cm^{-2}) than that of the voles (293 μW cm^{-2}).

The adaptive significance of these differences remains to be elucidated, but it may be related, at least partly, to the unique features of the species' habitats. Mole rats inhabit sealed underground tunnels under total darkness (Nevo, 1988); therefore, brief light exposure only occurs while mounding excavated material outside the tunnels. Under these extreme conditions of darkness, a low irradiance threshold would be critical as a unique adaptation to the restricted fossorial life. This adaptation is suggested to facilitate entrainment of physiological and behavioral parameters, such as thermoregulation and reproduction (Cooper et al., 1993a; Cooper et al., 1993b), to the external light–dark cycle even under light of very low intensity. The statistically non-significant effects of increasing light intensity (above 73 μW cm^{-2}) on urinary MEL and total urine production levels suggest a saturation of the mole rats' photoreception system at the higher intensities and thus intensities above this threshold have no effect on nocturnal MEL levels.

In contrast, voles required light of higher intensity to adjust daily variations in urine production rates. Voles are a semi-fossorial species that forage on the surface and nest inside burrows for protection from predation and unfavorable environmental conditions. Furthermore, this species is mainly nocturnal, but becomes diurnal during the cold winter months (Harrison and Bates, 1991). Therefore, the high irradiance threshold of social voles revealed in this study may have survival importance during foraging in daylight hours. Additionally, taking into account the significant effects of very dim light (<0.2 lx) on circadian responses in hamsters (Evans et al., 2008), the observed high threshold may also provide protection against marginal disturbances in the above-ground photoenvironment such as lightning, artificial light, sky glow, moonlight, etc. Otherwise, such 'noise' would most likely engender adverse effects on the adaptive entrainment of physiological and behavioral variables and thus compromise the survival of the species in its natural environment (Gorman et al., 2006; Evans et al., 2007).

In *S. ehrenbergi*, our results suggest that increases in irradiance are associated with decreases in melatonin concentration. Thus, high photophase light intensities apparently attenuated the melatonin rhythm and this effect was most evident under the highest intensity of 498 μW cm^{-2}. The high irradiance-induced suppression of melatonin rhythms observed here is inconsistent with other studies that reported an intense day/night difference under high intensities compared with low intensities (Griffith and Minton, 1992; Vera et al., 2005). This intensifying effect of photophase light was also detected in our study in the *M. socialis* irradiance group (Figs 2 and 3). Why we observed a suppressed response in urinary melatonin secretion in *S. ehrenbergi* is not obvious, but we postulate that very high light conditions are challenging to mole rats and may have provoked acute stress responses. Under these light-challenging conditions processes that are not directly related to immediate survival, such as melatonin synthesis and release, would be suspended. Nonetheless, this is an interesting result that requires additional research

Recently, we have provided unequivocal evidence that light at night (LAN) exposure acts as a stressor for voles acclimated to short photoperiods and significantly impaires the ability to use light signals for adjusting metabolic responses (Zubidat et al., 2007). The LAN-induced effects are likely mediated by the suppression of the typical nocturnal MEL rhythm. Because the results of the present study clearly demonstrated a positive correlation between increasing light intensity during the photophase and daily mean MEL levels of voles, it is of interest whether the effects of LAN could be repeated by exposing *M. socialis* to extremely low irradiance during the photophase.

In conclusion, our results demonstrate for the first time that light of increasing intensity robustly affects urinary MEL levels of social voles and 'blind' mole rats. These results suggest that the two species utilize the light–dark cycle equally to adjust changes in physiological parameters such as MEL and urine production levels. However, the two species exhibited differential responses to increasing light intensity during the photophase. Light of low intensity is likely to be more effective in modifying urinary MEL and urine production levels of the subterranean 'blind' mole rats, whereas for the same purpose the fossorial sighted social voles require light of greater intensity. Our data suggest that the different light threshold sensitivities of social voles and 'blind' mole rats are related to the specific light conditions in their natural habitats.

Perspective

One of the most noticeable transformations of industrialization has been the introduction of modern lighting at all hours of the day and night. In our day, lights of different characteristics are applied to illuminate our environment during both day and night hours. Health risks due to LAN exposure are expected to be an increasing problem and these adverse effects are likely to be accredited to the suppression of MEL rhythm. Our results indicate, however, that light quality during the photophase is also an important factor affecting rhythm components of MEL. Therefore, light source characteristics should be carefully considered before use in human environments or laboratory housing.

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