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DIFFERENTIAL EFFECTS OF PHOTOPHASE IRRADIANCE ON METABOLIC AND URINARY STRESS HORMONE CONCENTRATIONS IN BLIND AND SIGHTED RODENTS

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The effects of different photophase irradiance levels on the daily rhythms of energy expenditure (DEE, calculated from oxygen consumption, VO_2) and urinary metabolites of stress hormones in sighted (*Microtus socialis*) and blind (*Spalax ehrenbergi*) rodents were compared. Five groups of each species were exposed to different irradiance levels (73, 147, 293, 366, and 498 $\mu W/cm^2$) under short photoperiod (8L:16D) condition with constant ambient temperature $25 \pm 2°C$ for 21 days before assessments. As light intensity increased from 73 $\mu W/cm^2$, both species reduced DEE, especially among *M. socialis*. Cosinor analysis revealed significant ultradian rhythms in VO_2 of *M. socialis* with period length being inversely related to irradiance level. Conversely, in *S. ehrenbergi*, robust 24 h VO_2 rhythms were detected at all irradiances. In *M. socialis*, significant 24 h rhythms in urinary output of adrenaline were detected only at 293 $\mu W/cm^2$, whereas for cortisol, unambiguous rhythms were detected at 73 and 147 $\mu W/cm^2$. Distinct adrenaline daily rhythms of *S. ehrenbergi* were observed at 73 and 293 $\mu W/cm^2$, whereas this species exhibited significant rhythms in cortisol at 147 and 293 $\mu W/cm^2$. Changes in photophase irradiance levels affected stress hormone concentrations in a dose-dependent manner. There were significant negative and positive correlations of *M. socialis* and *S. ehrenbergi* stress hormones, respectively, with increasing irradiance. Our results indicate photophase light intensity is another environmental factor that can significantly affect entrainment of mammalian daily rhythms. Both low and high irradiance conditions can trigger stress responses, depending on the species' natural habitat. (Author correspondence: zubidat3@013.net.il)

Keywords Arginine vasopressin; HPA axis; Masking; Predation pressure; Retinal photoreceptors; Stress hormone metabolites; Subterranean

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INTRODUCTION

Temporal organization of biological functions is a well-established phenomenon among mammals (Duguay & Cermakian, 2009). Light exposure to retinal photoreceptors is by far the main effector of mammalian entrainment pathways of the central as well as peripheral circadian clocks (Pittendrigh, 1993; Quintero et al., 2003; Tosini et al., 2008). Rodents are exceptional among mammals in their adaptations to a variety of habitats with vastly different and challenging light characteristics. Principally, these adaptations involve the light-detecting system, and the diversity in illumination niches is reflected by large anatomical variation in retinal circuitry and projections (Peichl, 2005). In some rodent species, vision is a fundamental sense for survival; thus, their eyes are well developed, whereas other rodent species display different degrees of limited vision, ranging from mild to total blindness. Generally, the eyes of blind species have atrophied and the vestigial retina has lost its image-processing capabilities, but they remain fully functional in photoperiodic entrainment (Klerman et al., 2002; Němec et al., 2007, 2008).

The differences between sighted species, such as social voles *Microtus socialis*, and blind species, such as mole rats *Spalax ehrenbergi*, likely represent retinal anatomical adaptations to their fossorial and subterranean lifestyle, respectively (Harrison & Bates, 1991; Nevo, 1999). The retina of social voles expresses both image-forming photoreceptors (IFPRs) and non-image-forming photoreceptors (NIFPRs) for visual and photoperiodic responses. Mole rats have a latent retina that mainly expresses NIFPRs (Cernuda-Cernuda et al., 2002; Cooper et al., 1993). IFPRs represent the classic retinal rod and cone photoreceptors that include the photopigments rhodopsin and opsins located in the outer retina, whereas the NIFPRs are located in the inner retina and mainly include melanopsin (Kavakl & Sancar, 2002; Nayak et. al., 2007).

Previous studies on humans (Klerman et al., 2002; Lockley et al., 1997) and rodents (Freedman et al., 1999; Lucas et al., 1999) with retinal deficiency have suggested that IFPRs are essential for image processing capability, but not for entraining photoperiodic responses. Conversely, the NIFPRs, which form retinal ganglion cells (RGCs) carrying the photopigment melanopsin, play a central role in light signal detection (Hankins et al., 2008; Hattar et al., 2002; Panda et al., 2003). However, the involvement of both systems in photoperiodic regulation remains unspecified, and further studies are warranted (Fu et al., 2005).

Although light is the most reliable temporal cue for entrainment of the mammalian circadian system, the relevant properties of light for entrainment (i.e., irradiance, spectrum, and duration) have not been adequately addressed in past research, in particular the effects of light on circadian photosensitivity in non-laboratory rodents. Light irradiance is

an important characteristic of light and presents another potential ambient factor that could affect the entrainment of the circadian system via changes in pineal melatonin profiles that mediate photoperiodic responses (Berson, 2007; Lynch et al., 1981; Pévet et al., 2006). Wild animals have received little attention in irradiance studies compared with farm animals. From the studies that have investigated the effect of irradiance on melatonin profile and different physiological processes (e.g., activity, endocrinology, puberty), it appears that every species has its own unique sensitivity threshold (Brainard et al., 1986; Bronson, 1979; Griffith & Minton, 1992; Nelson & Takahashi, 1991; Piacsek & Hautzinger, 1974; Vilaplana et al., 1995). In general, these species differences appear to be associated with the animals' lifestyle; further, species-specific light sensitivity thresholds are likely adaptive by improving reproduction and survival (Kennaway & Wright, 2002; Ritter, 1994).

Daily variation in metabolic responses has been demonstrated in several rodent species, including *M. socialis*. Overall, metabolic rate is under photoperiodic control and coincides with the animals' activity patterns, in which high and low metabolic rates are measured during the active and inactive phases, respectively (Haim et al., 2008; Zubidat et al., 2007). Elaborate and precisely coordinated stress responses to virtually any change that would threaten the natural equilibrium between animals and their surroundings are highly conserved traits among vertebrates. Generally, these complex responses involve the activation of stress systems, mainly the sympatho-adreno-medullaty (SAM) system and the hypothalamic–pituitary–adrenocortical (HPA) axis that release catecholamines and corticosteroids, respectively, to the general circulating system (Axelrod & Reisine, 1984; Gavrilović & Dronjak, 2006; Sapolsky, 2002). Daily and seasonal variations in stress responses of both the HPA axis and SAM system, which are entrained by the environmental light/dark cycles, have also been broadly documented in mammal species, including members of the *Microtidea* family, such as *M. socialis* (Haus, 2007; Krame & Sothern, 2001; Nelson & Martin, 2007; Zubidat et al., 2008). In wild-type rodents, integrated and timed metabolic and stress responses are of significant survival value in natural habitats, where individuals need to allocate energy resources to appropriate avenues.

Although *M. socialis* and *S. ehrenbergi* are equally adept at utilizing photoperiodic changes to adjust daily responses in physiology and behavior (David-Gray et al., 1998; Goldman et al., 1997; Haim et al., 1983, 2005; Rado et al., 1991; Zubidat et al., 2007), they are at the opposite ends of the spectrum of habitat and retinal circuitry characteristics. Yet, there has been no direct comparison of light as the principal temporal input for the circadian clock of the two species. *M. socialis* and *S. ehrenbergi* provide natural models for comparing how both retinal anatomical and habitat differences affect daily physiological rhythmicity in response to various

intensities of light. In this regard, the study of the daily rhythmicity of these two species, occupying different lighting habitats, is of interest to comparative and physiological ecologists. Thus, we hypothesized that if *M. socialis* and *S. ehrenbergi* were challenged acutely with different illumination levels, then we would expect strong endocrine stress responses and appropriate metabolic modifications to meet these energetically demanding challenges. To test this hypothesis, we assessed the impact of photophase irradiance on daily energy expenditure (DEE, estimated by monitoring oxygen consumption, VO_2) and the urinary metabolites of the adrenaline (UMAdr) and cortisol (UMCort) stress hormones.

MATERIALS AND METHODS

Animals and Housing

For the study, 40 adult males of both social voles *M. socialis* (62 ± 7 g) and of mole rats *S. ehrenbergi* (256 ± 47 g) were used. Second- and third-generation *M. socialis* (3–4 months of age) were obtained from our breeding colony (Oranim, University of Haifa, Haifa, Israel) that was established from wild mating pairs. Individuals of *S. ehrenbergi* from the chromosomal superspecies 2n = 60 (Nevo et al., 2001) were captured in cultivated fields from around the Rehovot area (31°53′33.98″N 34°48′40.58″E) during winter and spring. Before the experiments started, *M. socialis* and *S. ehrenbergi* were caged separately in transparent polycarbonate cages (43 × 23 × 26 cm) layered with sawdust as bedding in an environmentally controlled room at ambient temperature (T_a) of 25 ± 2°C, relative humidity (RH) of 60%, and with 12 h L:12 h D photoperiod schedule (white light of 125 μW/cm^2). Before and during the experiments, Purina rodent 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 supplied *ad libitum*. Experiments were conducted with approval from the Ethics and Animal Care Committee of the University of Haifa and in accordance with the journal's guidelines for the care and use of animals in chronobiology research (Portaluppi et al., 2008). The research was conducted between June 2007 and May 2009 at the University of Haifa-Oranim.

Experimental Protocol

The research consisted of two experiments that were designed to examine the effect of irradiance increment on both the daily rhythms of VO_2 and UMAdr and UMCort in *M. socialis* and *S. ehrenbergi*. For this, five different groups of each species were exposed to one of five different light intensities (yellow = 586 nm) for at least 21 days before monitoring

VO_2 or collecting urine samples for analysis of the stress hormones. All experiments were conducted within a climatic room under short-day (SD) conditions of 8L:16D at $T_a = 25 \pm 2$°C, RH = 60%.

Daily Rhythms of VO_2

VO_2 was monitored using an open-flow computerized system capable of measuring levels simultaneously from five different metabolic chambers (2L in volume) for five consecutive days as previously described (Zubidat et al., 2007). Concentrations of O_2 in the dried air efflux from the metabolic chamber were monitored in 100 s time bins by an oxygen analyzer (Servomex Xentra 4100, Crowborough, UK) that interfaced with a computer utilizing Logal hardware and special software for viewing and analyzing collected data (MODCON systems, Wonderware InTouch 7,1,0,0; Tuchenhagen, Ireland, Ltd.). The metabolic chambers were placed inside a light-proof environmental incubator (LAB-Line EnvironETTE®, Dubuque, Iowa, USA) maintained at a constant regulated $T_a = 25 \pm 2$°C, RH = 60%, and SD conditions of 8L:16D. To regulate the amount of light emitted to the desirable level, incandescent lamps (n = 4; 40 w, OSRAM; Molesheim, France) were connected to a manually adjustable potentiometer (230V AC; Fetaya LTD; Rishon Le Zion, Israel). Lamps were installed ~30 cm above the chambers, and irradiance levels were measured within each chamber to establish a mean intensity level. Irradiance levels were measured using a calibrated handheld fiber optic spectrometer (AvaSpec-2048-FT-SDU, Avantes, Eerbeek, The Netherlands) while placing the light sensor at floor level in the center of the chamber. Five different mean light irradiances were established: 73 ± 15, 147 ± 4, 293 ± 7, 366 ± 16, and 498 ± 5 $\mu W/cm^2$. For reference, the average intensity of solar radiation in the region where the animals reside is ~1820 $\mu W/cm^2$ in the summer and ~479 $\mu W/cm^2$ in the winter (Acra et al., 1990).

Daily Rhythms of Stress Hormones

Urine Collection

Urine samples were collected from *M. socialis* and *S. ehrenbergi* at 4 h intervals for 28 h using a noninvasive procedure previously described (Zubidat et al., 2008). Urine samples were collected in a light-proof, T_a- and RH-controlled room (25 ± 2 °C and 60%, respectively) under 8L:16D. Light intensity inside the room was controlled with a similar electronic lighting dimmer circuit as descried above. The circuitry controlled eight yellow light lamps (40 w, OSRAM; France) positioned 60 cm apart and 30 cm above the animals' cages. At the end of the 21-day acclimation under a

given irradiance, animals were individually transferred to specially designed urine collection cages (48 × 38 × 21 cm) with an electropolished AISI 304 stainless steel 0.7 × 0.7 cm mesh floor (TECNIPLAST S.p.a; Buguggiate, Italy). Urine was collected in a plastic tray beneath the cages and was transferred at 4 h intervals to Eppendorf tubes using disposable glass pipettes. All urine collection cages were placed inside the same acclimation temperature-controlled room; thus, the animals were continuously exposed to the experimental conditions throughout the 28 h span of urine collection. Evaporative weight losses of the urine were negligible, because samples were collected frequently at a high relative humidity (60%) and all experienced the same marginal evaporation rate. Immediately after collection, each urine specimen was fractionated into two equal parts: one part for UMAdr and the other for UMCort determinations (the UMAdr aliquot was maintained at pH ~3 by adding 2–3 drops of hydrochloric acid [0.1N HCl]). The aliquots of both hormones were then directly preserved in a freezer at –25°C for further analysis.

Hormonal Analyses

UMAdr and UMCort concentrations were assessed using commercial enzyme-linked immmunosorbent assay kits (ELISA; IBL, Hamburg, Germany; Cat.-no.: RE59251 and RE52241, respectively). 20 µl and 10 µl duplicate aliquots for UMAdr and UMCort determinations, respectively, were analyzed by this method as previously described (Zubidat et al., 2008). The concentration of hormone in the sample was inferred from the fluorescence spectra at 450 nm, using a microplate ELISA reader (SunRise; Tecan, Grödig, Austria) and Magellan^{TM} data analysis software (Tecan). The intra- and inter-run precision had a coefficient of variation of 5.4% and 12.8% for UMAdr and 3.5% and 6.9% for UMCort, respectively. The analytical sensitivities for adrenaline and cortisol in urine samples were 0.3 ng/mL and 2 ng/mL, respectively.

Body Temperature Levels

The 40 w light lamps used in our experiments are expected to radiate thermal energy due to the high temperature of their incandescent tungsten filaments, and this could affect the animals' body temperature and consequently confound our results. To address this concern, we complementarily examined the effect of the 40 w incandescent lamps on body temperature (T_b) of the two species. T_b values of five individuals of each species were compared under two light treatments: 73 µW/cm^2 and 498 µW/cm^2. Animals were maintained individually in identical polycarbonate cages as in the irradiance experiments detailed above. Cages were placed 60 cm apart in a non-temperature controlled room. Five light

fixtures containing single 40 w incandescent bulbs were positioned 30 cm above the cages (~50 cm from the cage floor) and 60 cm apart. Rectal T_b values of the animals were recorded after an 8 h exposure (photophase length in our experiments) to either 73 μW/cm^2 or 498 μW/cm^2 as previously described (Zubidat et al., 2007). Additionally, the T_a inside the room was evaluated. The thermometer probe (THERM 2420-1L, Ahlborn Mess-und Reglungstechnik GmbH Holzkirchen, Germany) was placed under the fixture at the cage level and T_a was recorded after 5 min.

Statistical Analyses

The 100 s time bins of VO_2 measurements were compiled into 1 h time bins, and DEE was calculated by assuming an energy equivalent of 20.92 KJ/liter of O_2 utilized, as described by Speakman (2000). Values of urinary output of stress hormone metabolites were corrected for body mass and expressed as pg·(m·Lg)$^{-1}$. All data are expressed as mean ± one standard error (SEM) or 95% confidence interval (CI) of the mean. A two-way mixed-designed analysis of variance (TMANOVA) with repeated-measures on time and independent measures on irradiance was chosen to test for effects of time, irradiance, and time × irradiance interaction effects on daily rhythms of VO_2 and stress hormones in each species. The TMANOVA test was followed by a Student-Newman Keuls (SNK) post-hoc multi-comparison to assess differences between irradiance groups. One-way repeated-measure analysis of variance (RMANOVA) with Bonferroni post-hoc pairwise comparisons was completed to establish differences between timepoints when significant effects of time or interaction were detected by TMANOVA. Group mean differences and day–-night differences were also analyzed using Student's paired and unpaired *t*-test when appropriate. Pearson's correlation test was used to evaluate the relationship between increasing irradiance and DEE, mean UMAdr, or mean UMCort levels.

The described standard statistical analysis of the time series data were further complemented by a reiteration least squares fitting method, referred to as "single cosinor analysis" (Nelson et al., 1979; Refinetti et al., 2007). The cosinor method is used to detect and characterize a rhythm by approximating time series data with a cosine model by the method of least squares. We used a defined function with trial periods (τ) of 3–24 h or a fixed 24 h period for VO_2 or stress hormones, respectively, which describe the level (Y) of the variable at time (t):

$$Y(t) = Mesor + Amplitude \cdot \cos\left(\frac{2\pi \cdot (t + Acrophase)}{Period}\right)$$

where

- *mesor* is the estimated central value of the best fitting cosine curve approximating all data;
- *amplitude* is the oscillation range between the extremes of the best-fitting cosine curve approximating the time series data;
- *acrophase* is the timing of the maximum value of the best fitting cosine curve expressed as negative value (a delay from the phase reference) in degrees, with 360 °= 24 h and 0 ° set to local midnight (00:00) as the phase reference; and
- *period (τ)* is the length of one completed cycle of the best-fitting cosine approximating all data (i.e., 24 h).

The Jankins-Watt autoperiodogram for samples collected at regular intervals was used for periodicity analysis of the VO_2 data (Gouthiere et al., 2005). A significant rhythm was detected if the amplitude differed significantly ($p < 0.05$) different from zero. This was accomplished by F-test of variance explained by the straight line fit of the time series data versus that explained by the approximation of the cosine curve of a given τ. Additionally, the analysis also derives the so-called percentage rhythm (PR), which indicates the percent of variability accounted for by the cosine approximation. Results derived by the single cosinor analysis were then used to estimate the group mean mesor, amplitude, and acrophase parameters. The Bingham test (Bingham et al., 1982) was used to evaluate the effects of increasing irradiance on each of the rhythm's estimated parameters of each species. The cosinor analysis was performed with the TSA-Time Series Analysis Serial Cosinor 6.3 software package (Expert Soft Technology, Esvres; France; Gouthiere et al., 2005), whereas the SPSS 15.0.1 statistical software package (SPSS Inc., Chicago, Illinois, USA) was used to conduct all other typical statistical tests. Level of statistical significance was set at $p \leq 0.05$ and all statistical comparisons were two-tailed.

RESULTS

Daily Rhythms of VO_2

Graphs of VO_2 daily rhythms in both species after exposures to the five increasing photophase light irradiances are presented in Figure 1. In *M. socialis*, TMANOVA indicated highly significant effects of time ($F_{48,1680} = 3.25$, $p < 0.001$) and intensity ($F_{4,35} = 5.48$, $p = 0.002$) on VO_2 levels. Also, a significant interaction between the photophase intensity and time-of-day ($F_{192,1680} = 1.31$, $p < 0.005$) was revealed by ANOVA. SNK post-hoc complementation showed that mean VO_2 levels were significantly ($p < 0.05$) higher after exposure to the lowest irradiance (73 $\mu W/cm^2$) than highest level (498 $\mu W/cm^2$). Subsequently, the separated

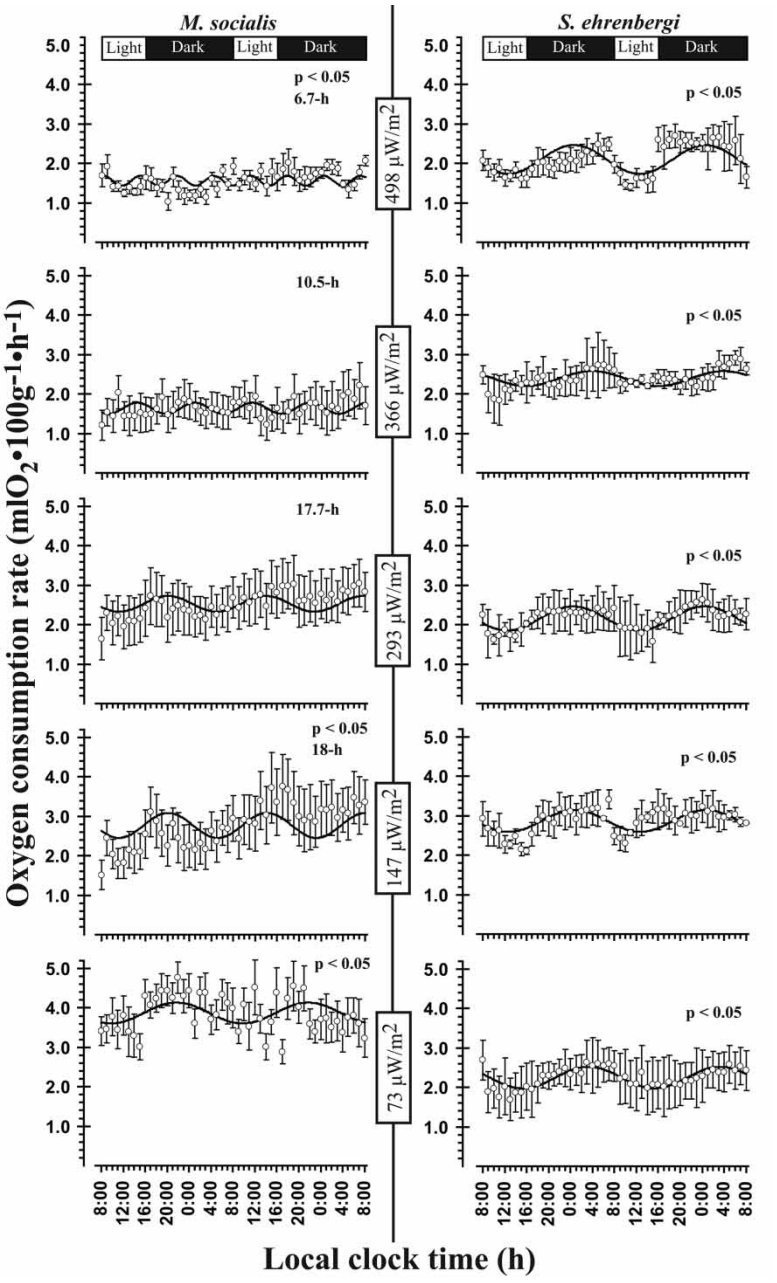

FIGURE 1 Daily rhythms of oxygen consumption measured over 48 h period, as a response to photophase light of various irradiance levels in *Microtus socialis* and *Spalax ehrenbergi* acclimated to short day conditions (16D:8L). Light irradiance levels are vertically designated in the middle of the figure. White and black horizontal bars represent photophase and scotophase alteration. $P < 0.05$ indicates significant rhythms with 24 h, unless otherwise indicated. Values are mean +/−SEM (white circles) of n = 8 per group. $P < 0.05$ from the non-zero amplitude test. The period length of the rhythms are of 24-h, otherwise the estimated length of a complete cycle is indicated. Black solid line illustrates the best least squares approximating cosine curve fitted to the entire data.

RMANOVA for the effect of time on VO_2 levels at each irradiance level revealed significant effects at 73 μW/cm^2, but not at the other higher intensities ($F_{48,336} = 2.35$, $p < 0.001$). In *S. ehrenbergi*, clear effects of time ($F_{48,1680} = 27.84$, $p < 0.0001$), intensity levels ($F_{4.35} = 8.03$, $p < 0.0001$), and interactions ($F_{192,1680} = 4.07$, $p < 0.0001$) were established. VO_2 levels in the 73 μW/cm^2 group differed significantly (SNK, $p < 0.05$) compared with those monitored for the other intensity groups. Consistently, the irradiance treatments group specific RMANOVA also revealed significant time effect on VO_2 levels at all irradiance conditions.

Significant day/night differences in energy expenditure (EE) levels in the 73 μW/cm^2 *M. socialis* group were evident (paired *t*-test: $t = -2.18$, df = 7, $p = 0.04$), but no differences existed in the other remaining groups. *S. ehrenbergi*, however, showed clear day/night differences at all irradiance groups, with higher levels during the scotophase compared with those estimated during the photophase (see Figure 2B). The estimated mean total DEE levels for the five irradiance groups of *M. socialis* and *S. ehrenbergi* differed significantly ($F_{4,35} = 7.61$ and 8.08, $p < 0.0001$, respectively) with highest levels at 73 and 147 μW/cm^2 (see Figure 2A). Likewise, DEE levels in the two species significantly decreased with light irradiance increment with larger negative correlation detected for *M. socialis* compared with *S. ehrenbergi* (Pearson's correlation: $R = -0.61$, $p = 0.0001$, and $R = -0.31$, $p = 0.04$, N = 40, respectively).

Results of the mean group cosinor analysis are presented in Table 1. A significant ($p < 0.05$) 24 h rhythm was detected only for *M. socialis* exposed to 73 μW/cm^2 irradiance during the photophase. For all other irradiance group, rhythms with $\tau < 24$ h (18, 17.8; 10.5, and 6.7 h) were detected by spectral analysis, of which only the 18 and 6.7 h τ rhythm amplitudes significantly differed from zero. Interestingly, estimations of rhythm τs in *M. socialis* were inversely associated with photophase irradiance power levels. In contrast, cosinor analysis detected significant 24 h rhythms for all *S. ehrenbergi* irradiance groups. Generally, mesor and amplitude levels in *M. socialis* decreased with increasing intensity, but significant differences were established only between mesor levels of the different irradiance groups. Comparisons of the rhythm estimates failed to establish significant differences in mesor or amplitude levels between the five different *S. ehrenbergi* irradiance groups. However, all VO_2 acrophases occurred during the third quarter (00:00–04:00 h) of the scotophase period, with significant delay (~3.5 h; $p < 0.05$) upon exposures to both 73 and 366 μW/cm^2.

Daily Rhythms of Stress Hormones

Figure 3 shows the urinary UMAdr responses to irradiance increment in both species. In *M. socialis*, TMANOVA revealed a main effect of time

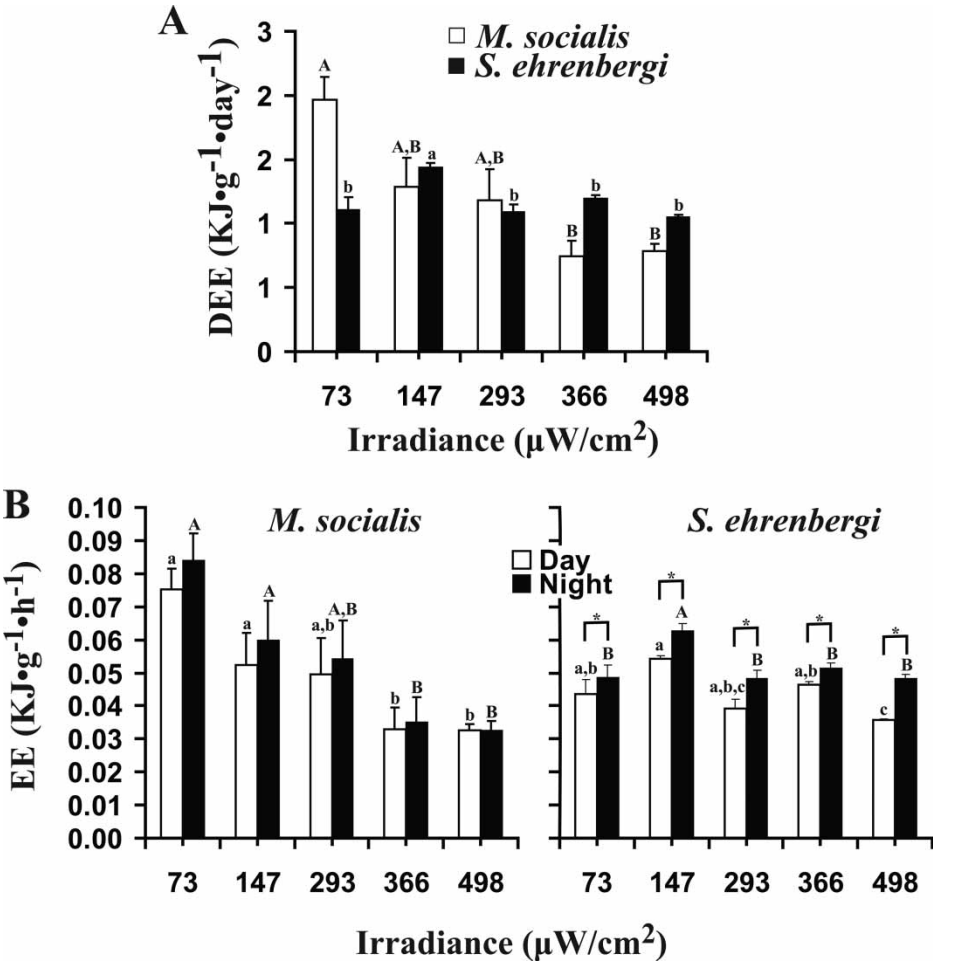

FIGURE 2 Mean +/− SEM (n = 8) total daily energy expenditure (DEE; A) and day/night differences in energy expenditure (EE; B) of *Microtus socialis* and moles rats *Spalax ehrenbergi* under short day photoperiod of different light irradiances during the photophase. EE was estimated from monitoring VO_2 by using the metabolic factor 20.92 KJ per liter of O_2 consumed. Significant differences between irradiance groups in each of the two species (One-way ANOVA, $p < 0.05$) are indicated by different letters. Significant day/night differences in each of the five irradiance groups are presented by asterisk (Paired *t*-test, $p < 0.05$).

course ($F_{6,180} = 2.51$, $p = 0.02$) and increasing irradiance ($F_{4,30} = 26.44$, $p < 0.0001$), but not time × irradiance interaction ($F_{24,180} = 0.49$, $p = 0.98$). Maximum effects of irradiance were observed at both the lowest and the highest levels, in which hormone levels were significantly (SNK; $p < 0.05$) elevated (~433 pg(mLg)$^{-1}$), compared with levels in the intermediate irradiance groups. However, significant time-related variations in UMAdr were detected only in the 293 µW/cm^2 group (RMANOVA; $F_{4,30} = 26.44$, $p = 0.98$) in which scotophase levels at 00:00 h (316.68 ± 26.9 pg(mLg)$^{-1}$) were

TABLE 1 Group mean cosinor analysis of daily rhythms in oxygen consumption under increasing light irradiance during the photophase of a short photoperiod

Irradiance ($\mu W/cm^2$)	Period (h)	Mesor ($mL \cdot 100g^{-1} \cdot h^{-1}$)	Amplitude ($mL \cdot 100g^{-1} \cdot h^{-1}$)	Acrophase (hh:min)	PR (%)	$F_{2,6}$; *p
M. socialis						
73	24	3.87[a] [3.74;3.99]	0.26 [0.08;0.44]	21:44 [18:52;00:40]	2.08	12.22; 0.02
147	18	2.77[b] [2.59;2.94]	0.32 [0.08;0.55]	14:10 [13:10;19:29]	1.74	4.98; 0.03
293	17.8	2.53[b]	0.2	13:18	0.80	2.99; 0.13
366	10.5	1.64[c]	0.14	01:43	0.67	1.52; 0.29
498	6.7	1.57[c] [1.50;1.63]	0.13 [0.04;0.21]	05:13 [04:31;06:04]	1.94	11.79; 0.02
S. ehrenbergi						
73	24	2.25 [1.90;2.59]	0.28 [0.20;0.35]	03:16[a] [02:13;04:20]	11.41	5.14; 0.0001
147	24	2.87 [2.83;2.91]	0.28 [0.21;0.33]	00:43[b] [00:04;01:28]	19.46	6.94; 0.0001
293	24	2.16 [2.12;2.21]	0.31 [0.24;0.37]	00:28[b] [00:17;01:28]	18.89	5.64; 0.0001
366	24	2.39 [2.35;2.42]	0.19 [0.14;0.25]	03:55[a] [02:46;05:03]	10.26	5.14; 0.0001
498	24	2.10 [2.06;2.14]	0.36 [0.31;0.42]	00:33[b] [00:02;01:09]	28.63	7.14; 0.0001

Values for mesor amplitude and acrophase are mean and 95% confidence interval (CI) of the group mean (CI values are not listed when $p > 0.05$). PR- Percentage rhythm, an estimate of the amount of variance explained by the iterative least squares fitting curve. *- the estimated probability of rejecting the null hypothesis (H_0 : Amplitude = zero). Different letters express significant differences between irradiance groups in the same species (Bingham test; $p < 0.05$).

notably elevated (SNK, $p < 0.05$) compared with photophase levels at 12:00 h (150 ± 15.89 $pg(mLg)^{-1}$). In *S. ehrenbergi*, UMAdr levels were affected only by irradiance conditions (TMANOVA; $F_{4,30} = 14.10$, $p < 0.0001$). Mean levels calculated for the highest irradiance group were about three-fold higher than in the lowest irradiance group.

The TMANOVA detected significant effect of time ($F_{6,180} = 5.75$, $p < 0.0001$), intensity of treatments ($F_{4,30} = 4.39$, $p = 0.01$), and interactions ($F_{24,18} = 3.43$, $p < 0.0001$) on UMCort levels in *M. socialis* (see Figure 4). Maximum increasing effects on UMCort levels were manifested at the lowest irradiance levels (90.66 ± 18.68 $pg(mLg)^{-1}$) that were about three-fold higher in comparison with levels at the highest irradiance treatment (29.73 ± 11.51 $pg(mLg)^{-1}$). Significant time-related variations were detected only in *M. socialis* exposed to either 73 or 147 $\mu W/cm^2$ (RMANOVA; $F_{6,36} = 8.90$ and 5.33, $p < 0.001$; respectively), whereas generally hormone levels of the urine samples obtained during the

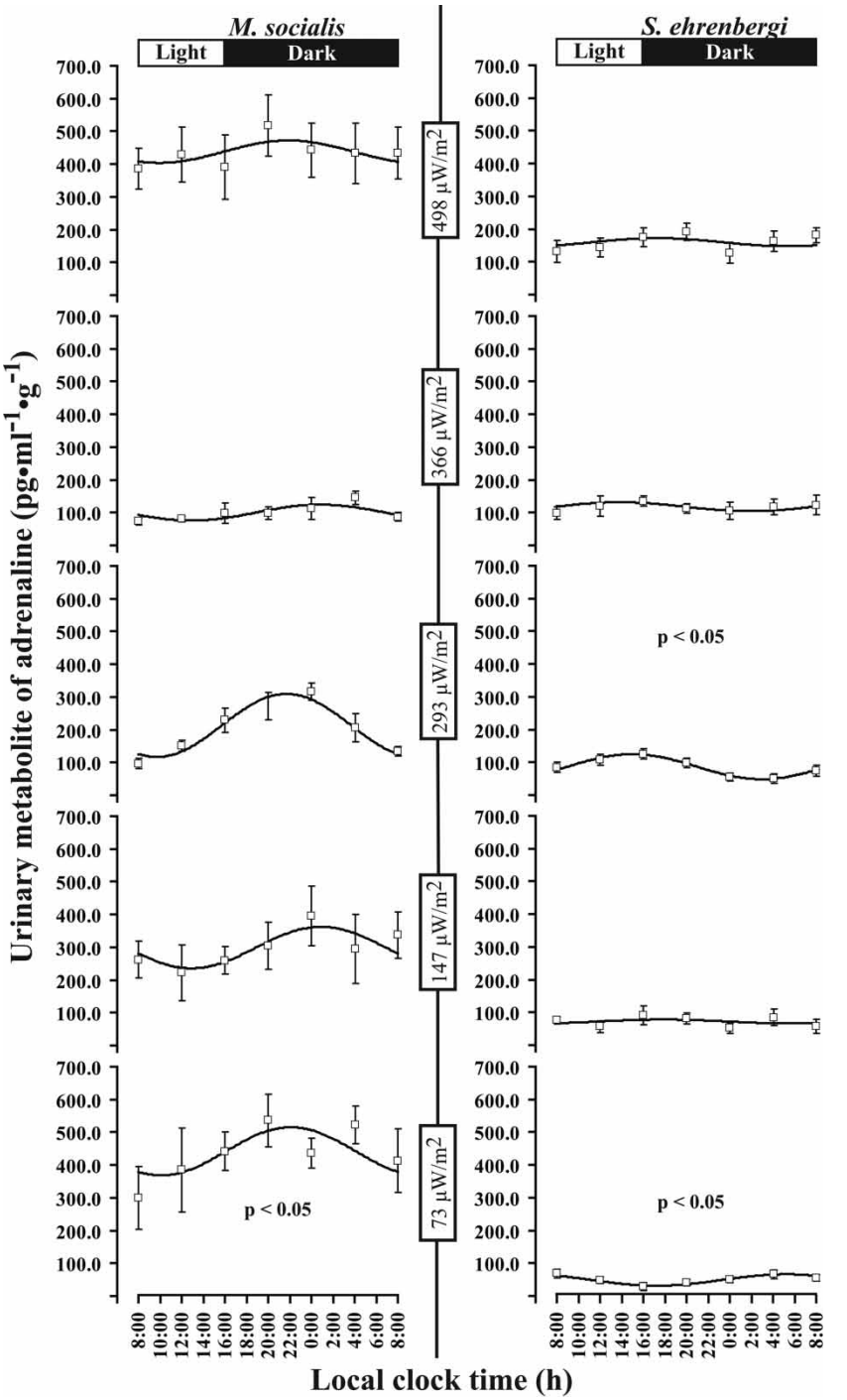

FIGURE 3 Daily rhythms of urinary adrenaline metabolite in response to light of five different photophase irradiances in short day acclimated *M. socialis* and *S. ehrenbergi*. All results are mean +/− SEM of n = 7. For more details see the legend of Figure 1.

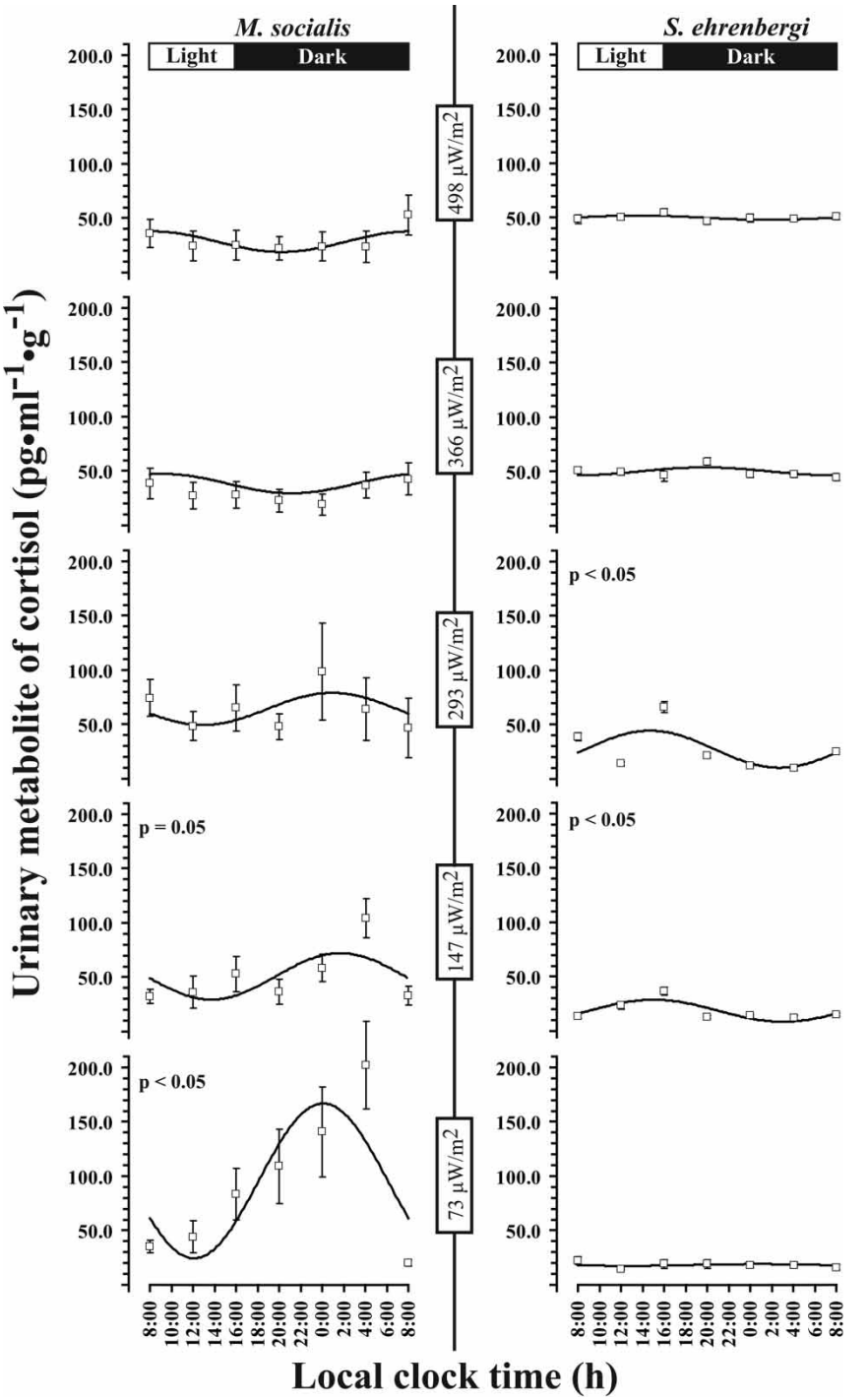

FIGURE 4 Daily rhythms of urinary cortisol metabolite in response to light of five different photophase irradiances in short day acclimated *M. socialis* and *S. ehrenbergi*. All results are mean +/− SEM of n = 7. For more details see the legend of Figure 1.

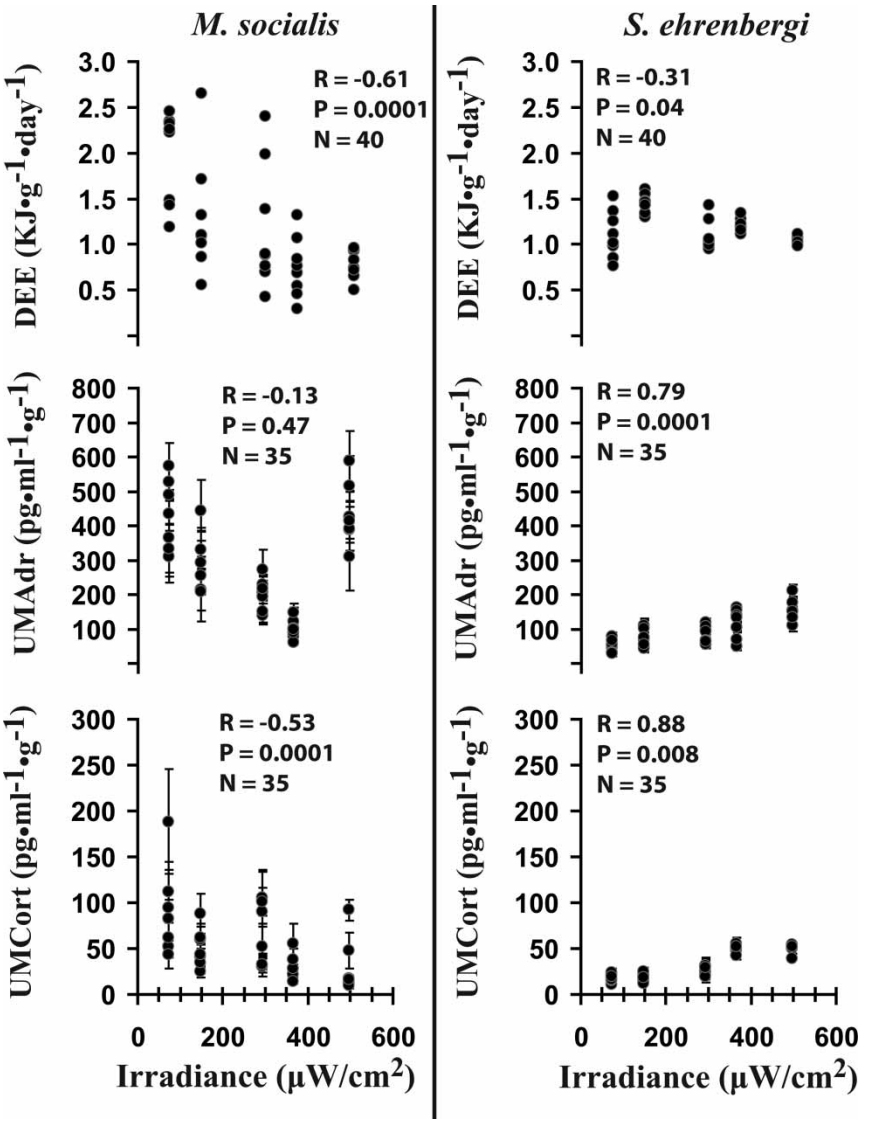

FIGURE 5 Correlation between change in photophase irradiance levels and change in stress hormone concentrations (adrenaline- UMAdr and cortisol- UMCort) in *M. socialis* and *S. ehrenbergi* under short day photoperiod conditions. *P*- the probability value for the pearson's correlation coefficient (*R*).

scotophase surpassed those obtained during the photophase. Similarly, UMCort daily variations of *S. ehrenbergi* were affected by time ($F_{6,180} = 24.87$, $p < 0.001$), intensity ($F_{4,30} = 90.73$, $p < 0.001$), and interactions ($F_{24,180} = 12.14$, $p < 0.001$). Overall, SNK post-hoc analysis indicated that elevated hormone levels were measured in the high (366 and 498 µW/cm^2) compared with the low (73 and 147 µW/cm^2) irradiance exposure

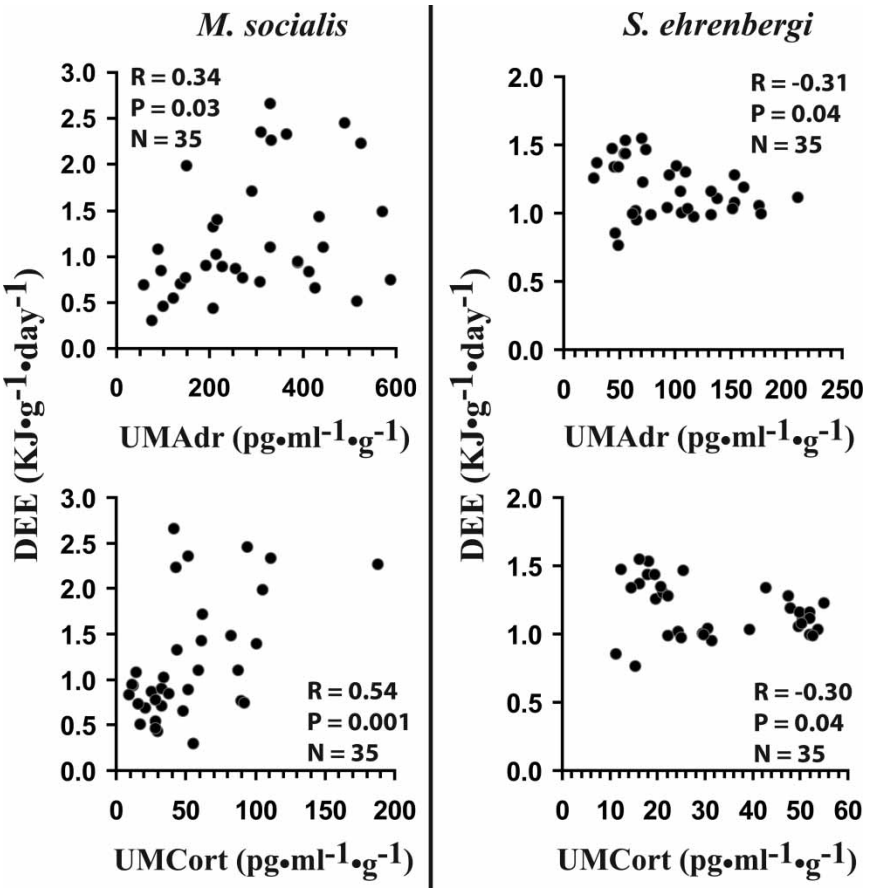

FIGURE 6 Correlation between change in stress hormone concentrations (adrenaline- UMAdr and cortisol- UMCort) and daily energy expenditure levels (DEE) in *M. socialis* and *S. ehrenbergi* under short day photoperiod conditions. *P*- the probability value for the pearson's correlation coefficient (*R*).

groups. When the specific effect of time elapsed was analyzed for each irradiance group, significant variations in hormone levels were verified for both the 147 and 293 $\mu W/cm^2$ photophase exposures, but no significant effects were detected among the remaining groups.

As shown in Figure 5, Pearson's coefficient confirmed that changes in the stress hormones of *M. socialis* showed a negative relationship with changes in photophase irradiance. Significant though lower correlations were established for UMAdr and UMCort ($R = -0.13$, $p = 0.47$ [UMAdr] and $R = -0.53$, $p = 0.0001$ [UMCort], N = 35, respectively; see Figure 5). In contrast, a high and positive correlation between changes in stress hormones and changes in photophase irradiance was found in *S. ehrenbergi* ($R = 0.79$, $p = 0.0001$ [UMAdr] and $R = 0.88$, $p = 0.01$ (UMCort), N = 35, respectively; see Figure 5). There were significant positive correlations

TABLE 2 Group mean cosinor analysis of urinary metabolites in adrenaline under increasing light irradiance during the photophase of a short photoperiod

Irradiance ($\mu W/cm^2$)	Mesor ($pg \cdot mL^{-1} \cdot g^{-1}$)	Amplitude ($pg \cdot mL^{-1} \cdot g^{-1}$)	Acrophase (hh:min)	PR (%)	$F_{2,5}$; $^*p$
M. socialis					
73	442[a]	73.5	22:04	5.79	2.51; 0.25
147	299[b]	63.3	00:52	4.59	1.49; 0.34
293	213[b] [190;236]	96.4 [65.2;128]	21:40 [20:20;23:04]	45.91	17.38; 0.0001
366	100[c]	24.3	00:49	7.98	7.15; 0.15
498	438[a]	34.1	21:52	1.35	2.22; 0.75
S. ehrenbergi					
73	48.7[a] [40.4;57.1]	17.5 [5.71;29.2]	05:12 [02:21;08:04]	16.5	9.55; 0.02
147	72.2[b]	6.05	17:48	0.71	0.12; 0.85
293	86.6[b] [75.3;97.8]	38.2 [21.4;54.9]	18:24 [15:08;21:36]	31.39	26.22; 0.0002
366	118[c]	13.6	13:48	2.11	1.7; 0.61
498	160[d]	12.4	16:44	1.36	2.03; 0.73

- For more details see the legend of Table 1.

between DEE levels and both UMAdr and UMCort concentrations ($R = 0.34$, $p = 0.03$ and $R = 0.54$, $p = 0.001$, N = 35, respectively; see Figure 6) in the *M. socialis* irradiance groups. Contrary to this finding, DEE levels in *S. ehrenbergi* irradiance groups were negatively associated with both UMAdr and UMCort ($R = -0.31$ and -0.30, $p = 0.04$ for both, N = 35, respectively; see Figure 6).

In general, results of the cosinor analysis were consistent with those of the ANOVA. In *M. socialis*, a significant ($p < 0.0001$) 24 h rhythm in UMAdr was validated only in the 293 irradiance group (see Table 2). Significant 24 h rhythms in UMCort were also detected at 73 ($p < 0.0001$) and 147 $\mu W/cm^2$ ($p < 0.05$) photophase irradiance exposures, but not in the other irradiance groups (see Table 3). All rhythm estimates for UMAdr were affected by irradiance increment, but only the mesor showed clear changing patterns; higher mesor levels at extreme levels compared with intermediate levels. Changes in the amplitudes and acrophases showed no clear direction with increasing photophase irradiance. In *S. ehrenbergi*, significant 24 h oscillations in UMAdr were established only at 73 and 293 $\mu W/cm^2$ ($p < 0.002$ and 0.0002, respectively), while for UMCort, significant 24 h rhythms were estimated only at 147 and 293

TABLE 3 Group mean cosinor analysis of urinary metabolites in cortisol under increasing light irradiance during the photophase of a short photoperiod

Irradiance (μW/cm^2)	Mesor (pg·mL^{-1}·g^{-1})	Amplitude (pg·mL^{-1}·g^{-1})	Acrophase (hh:min)	PR (%)	$F_{2,5}$; *p
M. socialis					
73	95.6[a] [72.4;119]	71.2 [37.3;105]	00:04 [23:51;01:44]	28.24	9.21; 0.0001
147	50.8[b] [39.5;62.1]	21.3 [4.34;38.3]	01:39 [01:38;04:39]	12.2	7.18; 0.05
293	64.2[a,b]	14.8	00:53	2.24	0.39; 0.59
366	38.5[b,c]	8.93	01:00	2.91	0.53; 0.51
498	28.4[c]	9.74	21:44	3.86	1.02; 0.40
S. ehrenbergi					
73	18.3[a]	0.80	00:36[a]	0.44	0.35; 0.90
147	18.7[a] [16.5;21.0]	10.1 [6.37;13.4]	15:00[b] [13:52;16:12]	44.49	15.42; 0.0001
293	27.4[b] [22.4;32.3]	17.0 [9.72;24.4]	14:40[b] [13:08;16:12]	32.31	93.33; 0.0001
366	50.2[c]	3.67	19:44[b]	6.97	5.36; 0.19
498	50.0[c]	2.02	13:36[b]	2.96	4.28; 0.50

- For more details see the legend of Table 1.

μW/cm^2 ($p < 0.0001$; see Tables 2 and 3). Comparison of the rhythm parameters revealed that the UMAdr and UMCort stress hormone patterns of *S. ehrenbergi* in the five irradiance groups changed as irradiance increased, but only the mesor showed a significant and clear direction of effect. Mesor levels of both hormones increased with increasing intensity, whereas amplitude and acrophase showed no clear direction of effect. In each of the two species, the highest percentage rhythm values for the stress hormone rhythms were found in the 293 μW/cm^2 irradiance group (see Tables 2 and 3).

Body Temperature Levels

Under non-temperature controlled conditions, a slight upward thermal shift (1.72 ± 0.25°C) was recorded in the mean T_a at the cage level in response to an increase in irradiance level of the five 40 w incandescent bulbs from 73 μW/cm^2 to 498 μW/cm^2. Nonetheless, we found that the light treatments had no significant effect on mean T_b of *M. socialis* and *S. ehrenbergi* at either 73 μW/cm^2 or 498 μW/cm^2. In *M. socialis*, the mean T_b under 73 μW/cm^2 was 39.04 ± 0.27°C, whereas increasing

the irradiance levels to 498 $\mu W/cm^2$ had no effect on mean T_b of *M. socialis*, which was 38.72 ± 0.11°C (Paired *t*-test: $t = -1.05$, $df = 4$, $p = 0.40$). Moreover, increasing the irradiance level from 73 $\mu W/cm^2$ to 498 $\mu W/cm^2$ showed no significant effect on the mean T_b of *S. ehrenbergi* (35.66 ± 0.22°C and 36.06 ± 0.22°C, respectively; Paired *t*-test: $t = -1.55$, $df = 4$, $p = 0.20$).

DISCUSSION

Daily Rhythms of VO_2

We report here a prominent effect of photophase light intensity on metabolic responses of two species with different visual systems and lighting conditions in their natural habitats. In the two species, daily rhythms of VO_2 were affected by changes in photophase irradiance levels (see Figure 1), and this was manifested as decreased levels in DEE with increased photophase irradiance (see Figure 2).

In vertebrates, there is substantial experimental evidence indicating that locomotor activity and EE are closely and positively interrelated (Bennett & Ruben, 1979; Taylor et al., 1982). Reduced locomotor activity in response to nocturnal bright-light conditions was previously reported for several rodent species (Karmer & Birney, 2001; Kotler, 1984; Vásquez, 1994). Furthermore, the activity of prairie voles, *Microtus ochrogaster*, was more pronounced in the absence of light than in the presence of brighter light conditions (Getz, 2009).

The molecular mechanism of the circadian clock regulating entrainment to light exposure is still unclear, but there is increasing evidence that the clock *Period* (*Per*) gene family plays a significant role in the resetting of the clock in diurnal and nocturnal mammalian species. In nocturnal rodents, *Per1* and *Per2* expression in the suprachiasmatic nucleus (SCN) increases during the photophase and decreases during the scotophase. *Per1* and *Per2* expression is instantly induced by light exposure (Dardente et al., 2002; Wilsbacher et al., 2002). Interestingly, in diurnal species, such as *Octodon degus* and *Arvicanthis ansorgei*, light exposure also induces upregulation of *Per1* and *Per2* expression, but only during the subjective night, as in their nocturnal counterpart species (Koch et al., 2009; Ramanathan et al., 2009). These comparable responses of diurnal and nocturnal species to light exposure suggest the differences between the two circadian activity patterns may reflect direct masking effect of light rather than a central effect on the SCN (Erkert, 2008).

In our study, the reported negative relation between photophase irradiance and DEE may also reflect a direct masking effect of metabolic and/or behavioral responses in *M. socialis* and *S. ehrenbergi*. As suggested

previously, light can either positively or negatively regulate masking effects on behavioral patterns of rodents, depending on its power and the animal's temporal niche (Erkert, 2008; Mrosovsky, 1999; Mrosovsky & Hatter, 2005). In nocturnal rodents, bright-light exposure attenuates activity levels (negative masking; Li et al., 2005; Mrosovsky et al., 2001) in a dose-dependent way (Mrosovsky, 1994). This masking effect can be mediated over extended periods of time by the NIFPRs, including melanopsin (Mrosovsky & Hatter, 2003). The involvement of IFPRs in irradiance-induced negative masking was also previously suggested by Thompson et al. (2008), where IFPRs are likely to be involved to some extent in confining light-mediated negative masking responses (Mrosovsky & Thompson, 2008). Nevertheless, our study showing increased irradiance-induced reduction of DEE rhythms in *M. socialis* and *S. ehrenbergi* is consistent with negative masking. Furthermore, the suggested dose-dependent masking effects of irradiance could also account for the ability of *M. socialis* to change its predominant temporal organization from nocturnal to diurnal during relatively dim winter days (Levy et al., 2007; Mrosovsky & Hatter, 2005; Redlin & Mrosovsky, 2004). The underlying mechanism by which light promotes its masking effects in mammalian species is complex and remains largely unknown (Redlin, 2001), and further studies are needed to clarify this issue.

Spectral analysis showed *M. socialis* responded to irradiance by shortening the τ of its VO_2 rhythm (see Figure 1). At the lowest irradiance (73 $\mu W/cm^2$), animals exhibited a 24 h oscillation; the τ decreased to 6.7 h as irradiance increased to the highest intensity (498 $\mu W/cm^2$). Ultradian patterning of VO_2 throughout the 24 h day in response to incremental increases in light intensity was also documented for daily activity in captive leaf-eared mice, *Phyllotis xanthopygus* (Karmer & Birney, 2001).

Ultradian rhythms in activity are a genuine and widespread feature among the genus *Microtus* (Halle, 2000), and high-frequency activity patterns have also been documented for *M. socialis* (presented in Benjamini, 1989, as *M. guentheri*). More recently, ultradian rhythms with a mean τ of 3.5 h in metabolic responses have also been described in our laboratory for *M. socialis* acclimated to short-day conditions (Zubidat et al., 2007). In addition, a direct link between ultradian rhythms in activity, outside the underground refuges, and metabolic rate has been reported in the root vole *M. oeconomus* (Gębczyński, 2006). Together, the results of these studies suggest that the observed irradiance dose-dependent effect on shortening the τ of VO_2 (i.e., as ultradian) rhythms of *M. socialis* is likely to be sustained by similar frequency domains in locomotor activity over the 24 h. Therefore, the coupling between DEE and activity in *M. socialis* could reflect an adaptive ecological response to balance-challenging conditions of predation risk and frequent food demands in its natural environment.

Changes in activity level in response to increased light intensities have been suggested to improve survival of small rodents in the face of predation by diurnal and nocturnal raptorial birds (Clarke, 1983; Getz, 2009; Karmer & Birney, 2001). Foraging above ground under bright-light conditions could endanger the life of prey, because under these conditions, the capture success of visually hunting predators could be improved. *M. socialis* is a semi-fossorial species that forages on the surface and nests inside burrows for protection from predation and unfavorable environmental conditions. Although it is a nocturnal species, it can display diurnal activity during low temperatures and overcast winter days (Harrison & Bates, 1991); thus, the reduced metabolic rates observed here in *M. socialis* may be an adaptive behavioral response to predation pressure.

The solitary *S. ehrenbergi* exists in complex subterranean networks of tunnels and chambers, in which it forages for roots and tubers (Nevo, 1988). Consequently, *S. ehrenbergi* is exposed to less predation pressure than *M. socialis*, and this could provide some explanation for the differential responses in DEE observed between the two species. In *M. socialis*, the correlation was −0.61 between DEE and irradiance level, whereas in *S. ehrenbergi* the correlation was only about half this value (see Figure 5). Furthermore, the ultradian rhythms observed for *M. socialis* are most likely to be a compensatory response to the decreased metabolic rates. On the one hand, the animal escapes potential predators by reducing its activity, while on the other hand it still needs to forage. Frequent bursts of activity, manifested as ultradian rhythms, during the night could allow sufficient foraging while reducing surface exposure to predators. Additionally, the ultradian activity patterning would restrict the prey animal activity close to the burrow openings to allow a rapid escape if warranted.

Our results also demonstrated that the sighted and the blind species are equally capable of detecting light of different intensities and respond accordingly by adjusting their VO_2 daily rhythms. Furthermore, the ability of the blind *S. ehrenbergi* to use as low as 73 $\mu W/cm^2$ of light irradiance to regulate daily responses in metabolic rates was not predicted. Thus, our results provide direct evidence that *S. ehrenbergi* have sensitive irradiance detection photoreceptors, and these are most likely to be responsible for light perception and circadian entrainment in this blind species.

Our present results showed that *M. socialis* respond to photophase irradiance increment by decreasing both mesor and the τ length of the VO_2 rhythm, whereas the amplitude remained statistically unchanged and no clear changes in the direction of the acrophase were apparent. Photophase irradiance also affected VO_2 rhythm estimates of *S. ehrenbergi*, but in comparison with *M. socialis* mesor levels and τ were not affected as

photophase irradiance increased. The best percentage rhythm values (i.e., the best approximation of the cosine 24 h model) in *S. ehrenbergi* were found for rhythms whose acrophase occurred between 00:00–01:00 h (see Table 1). These differential responses to increased photophase light intensity between *M. socialis* and *S. ehrenbergi* are likely related to different retinal photoreceptor projections to the circadian clock within the hypothalamus, such as the image and non-image forming photoreceptors (IFPRs and NIFPRs, respectively).

Urinary Stress Hormone Responses

Recently, we demonstrated light-at-night (LAN)-induced stress responses in *M. socialis* under SD conditions (Zubidat et al., 2007). Our present results also suggest that atypical change in photophase light conditions may be as efficacious as LAN in boosting stress responses in *M. socialis*, as well as in the blind *S. ehrenbergi*. To the best of our knowledge, this is the first study to show that photophase light conditions can also act as a stressor, resulting in increased urinary metabolites of stress hormones in non-laboratory rodent species. The photophase irradiance-induced stress responses of *M. socialis* and *S. ehrenbergi* were in opposite directions and within various amplitudes to irradiance exposure levels. Additionally, the amplitude of response was greater in the sighted *M. socialis* compared with the blind *S. ehrenbergi* (see Figures 3 and 4).

Non-domesticated animals are suggested to be especially sensitive to abnormal changes in their internal and external environments. Deviations from the optimal environment generally provoke stress responses that directly allocate energy to critical physiological and behavioral functions to maximize survival and return stability to homeostasis (Goldstein, 2003; Sapolsky, 2002). In its habitat, *M. socialis* is typically exposed to higher light intensities than *S. ehrenbergi*. Therefore, exposure to extremely low and high light intensities may disrupt the standard photo-environment, in which enhanced stress responses are expected. As for *S. ehrenbergi*, which exist in total darkness in underground burrows, increased light intensity is considered to be a major environmental perturbation resulting in the acute activation of the SAM system and HPA axis, causing a massive release of adrenaline and cortisol, respectively.

In both species, stress hormone levels were higher during the scotophase (activity span) in comparison with the photophase (rest span). The fundamental 24 h harmonic domain approximated here for UMAdr and UMCort (see Figures 3 and 4) is in agreement with substantial earlier studies that have described distinct circadian rhythms in stress hormones in rodent species (Ahlers et al., 1999; Albers et al., 1985; Atkinson & Waddel, 1997; Chacón et al., 2005; Sudo & Miki, 1995). These circadian rhythms in stress hormones are most likely regulated by the master

circadian clock, suprachiasmatic nuclei (SCN) within the hypothalamus (Challet, 2007). Overall, light information is transduced into neural signals and projected to the SCN by photoreceptor retinal circuits. The pineal gland completes this photo-transduction pathway by generating the circadian melatonin (MEL) rhythm characterized by high scotophase and low photophase levels. The pineal MEL rhythm reliably reproduces the environmental light/dark cycle and in this manner serves as an indoledial clock for measuring the passage of time and scheduling biological functions, including endocrine responses (Pévet et al., 2006; Wilkinson, 2008).

Accordingly, the differential photophase irradiance-induced rhythmic responses in stress hormones of *M. socialis* and *S. ehrenbergi* may reflect changes in MEL rhythm. Indeed, we recently reported that incremental increase in photophase irradiance inversely affected MEL levels (evaluated by its major urinary metabolite 6-sulfatoxymelatonin) in *M. socialis* and *S. ehrenbergi*, thus having a negative correlation in the former species and positive correlation in the latter species (Zubidat et al., 2009). The precise mechanism of MEL action in regulating the observed stress responses is not clear; however, we assume that the neurotransmitter arginine vasopressin (AVP) is a potential candidate that mediates stress responses of *M. socialis* and *S. ehrenbergi*. First, the existence of dual mechanisms of stimulation and inhibition of HPA axis modulation by the SCN has been suggested to involve different AVP neural projections (Kalsbeek et al., 2006). Second, daily rhythms of AVP (Windle et al., 1992) and MEL (Reiter, 1993) oscillate within an inverse phase pattern that is the result of an inhibitory relationship between the two hormones (Juszczak et al., 2007). Third, increased photophase irradiance levels promote reciprocal effects on urinary MEL concentrations in *M. socialis* and *S. ehrenbergi*, having increased levels in *M. socialis* and decreased levels in *S. ehrenbergi* (Zubidat et al., 2009). Finally, the present results showed that stress responses in *M. socialis* and *S. ehrenbergi* decreased and increased, respectively, with increasing photophase irradiance. Taken together, we suggest that the increased photophase irradiance-dependent-induced MEL levels in *M. socialis* could have attenuated the stress responses via imposing an inhibitory signal on AVP daily rhythms. In *S. ehrenbergi*, however, the situation is reversed; the increased photophase irradiance-induced decrease in MEL levels could elevate stress responses by abolishing the inhibitory effect on AVP daily rhythms. Because of the complexity of the interconnected neural projections involved in controlling adaptive stress responses (Hermana & Cullinan, 1997), we cannot exclude the involvement of other circuits in mediating the irradiance effects on stress responses in *M. socialis* and *S. ehrenbergi*.

The results of the present study showed that daily variation in stress hormones differed between *M. socialis* and *S. ehrenbergi*, in which higher

daily amplitude, mesor levels, and later acrophase occurrences (around mid-scotophase) were detected in the former species (see Tables 2 and 3) for all experimental groups. Accordingly, *M. socialis* when compared with *S. ehrenbergi* appears to be much more sensitive to changes in photophase irradiance levels as indicated by the measurable variations in UMAdr and UMCort levels. Furthermore, cosinor analysis revealed that the highest percentage rhythm values of stress hormones in *M. socialis* and *S. ehrenbergi* were obtained in the moderate irradiance groups (293 $\mu W/cm^2$) in which highly significant ($p < 0.0001$) effects were detected (see Tables 2 and 3). This result may imply that 293 $\mu W/cm^2$ of photophase light irradiance is the most effective level, among those evaluated here, for circadian entrainments in *M. socialis* and *S. ehrenbergi*. Regarding *M. socialis*, this finding is of significance if LAN exposure would be applied in agricultural systems as a possible novel solution for rodent pest problems, as previously suggested (Haim et al., 2004; Zubidat et al., 2007).

Regarding *M. socialis*, our results are consistent with the well-established metabolic effects of catecholamines and glucocorticoids (Silva, 2006) as DEE levels changed in direct relation to the concentrations of both UMAdr and UMCort. Contrary to our prediction, change in DEE of *S. ehrenbergi* showed no direct association with UMAdr or UMCort concentrations; instead, a negative relation was detected between the two variables. The fact that increased circulating levels of these hormones have no enhanced metabolic effects may be, at least in part, the outcome of lowered response to the hormone mediated by light-masking-induced down-regulation of its specific receptor at the cellular level. The attenuated metabolic response of *S. ehrenbergi* could also reflect a physiological adaptation to life in underground burrows, in which an increase in VO_2 would manifestly increase hypercapnic and hypoxic respiratory conditions, and under these conditions the animal's physiology may be adversely affected.

Body Temperature Levels

Although it is expected that incandescent light bulb release ~90% of their power as thermal energy, our results indicted that under non-temperature controlled conditions, the five 40 w incandescent light bulbs at 498 $\mu W/cm^2$ radiated heat and generated only ~1.72°C thermal differential at the animal cage level compared with the lowest irradiant level (73 $\mu W/cm^2$). Similarly, it was demonstrated previously that five 100 w incandescent light bulbs installed 18 cm above the experimental floor raised the T_a (measured 3 cm above the floor) by only ~3°C (Godsil & Fanselow, 2004). In our experiments, we installed much lower wattage light bulbs (40 w), fixed 30 cm above the cages (~50 cm above the cage floor), and their complete irradiance power (~700 $\mu W/cm^2$) was not

actually fully exploited under the highest irradiance level (498 ± 5 μW/cm^2). Importantly, the recorded slight thermal differential in our experiment exerted no significant effect on the mean rectal T_b of *M. socialis* and *S. ehrenbergi*. This response was expected, because the two species are equally competent in regulating their body temperature over a wide range of ambient temperature (Banin et al., 1994; Nevo & Shkolnik, 1974).

It is important to note that the complementary measurements of both T_a and T_b were performed under non-temperature controlled conditions, whereas our original irradiance experiments were conducted in an environmentally controlled room having an ambient temperature of 25 ± 2°C. Undoubtedly, we believe that the heat emission from the 40 w incandescent lights in our experiments did not reach critical levels to influence ambient temperature regulation inside the climatic room, including at the cage level. Therefore, it is tenable to conclude that the adaptive homeostatic control of body temperature in *M. socialis* and *S. ehrenbergi* was not critically challenged at all irradiance levels. In conclusion, our results strongly suggest the irradiance-induced differences in metabolic and stress responses between the two species are directly related to light power changes.

CONCLUSIONS

Photophase light quality exerts significant effects on entrainment physiology of *M. socialis* and *S. ehrenbergi*, as daily rhythms in metabolic and stress responses were altered with exposure to different photophase irradiance levels. The two rodent species responded differently in amplitude and direction to changes in photophase irradiance. Interestingly, low-light conditions during photophase triggered stress responses in the sighted *M. socialis*, whereas in the blind *S. ehrenbergi*, intense stress responses were elicited only at high-light exposures. These differences could be attributed to differences in the retinal phototransduction pathways that have been strongly shaped by local habitat conditions over millions of years. It is worth noting that our study evaluated the effects of monochromatic yellow light, and currently our laboratory is intensively engaged in evaluating the effects of other wavelength stimuli.

PERSPECTIVES

The effects of environmental light exposure on virtually all aspects of mammalian lives, including physiology, behavior, and ecology, have been the focus of considerable research during the past century (Navara & Nelson, 2007). In humans, LAN exposure is increasingly becoming recognized as a major health problem, particularly in modern urban

environments; many health risks (e.g., cancer) from LAN-induced circadian disturbances have been supported by several studies (Kloog et al., 2008; Srinivasan et al., 2008; Stevens et al., 2007). Hence, our results suggest that the light conditions during the photophase can be equally effective as during the scotophase in provoking circadian system dysfunction. Therefore, light properties should be properly characterized, before being practically applied as indoor lighting fixtures. Finally, it remains to be shown whether the photophase and scotophase light-induced circadian disruption are conveyed by the same or alternative visual pathway circuits.

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